STUDIES ON RAT INTESTINAL SUCRASE

by

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled "Studies on Rat Intestinal Sucrase" submitted by Donald George Ralph Blair, B. Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

An investigation was carried out to determine the localization of intestinal sucrase in the small intestine of the rat, and to elucidate information about the action of the enzyme and about the various factors affecting its activity.

The kinetics of the action of the enzyme were studied. It was found that the optimum pH was 6.25 ± 0.15 and that the optimum substrate concentration was 0.12 M (4.1%) to 0.5 M (17.1%) sucrose. The Km value for intestinal sucrase was 0.0128 M, while the activation energy, μ, was 10,100 calories per mole between 5° and 37°C. At a substrate concentration of 0.161 M (5.5%) sucrose, the reaction was zero order, and the rate of reaction was proportional to the enzyme concentration over the range studied.

Intestinal sucrase was found associated mainly with the first half of the small intestine of normal adult male rats, and the enzyme activity in the remaining portions of the small intestine decreased almost to zero at the ileocolic end. A survey of other tissues of normal adult male rats revealed a trace of sucrase activity in the large intestine only. Intestinal sucrase activity varies with the age of the animals, but no difference was observed between the enzyme levels of male and female rats of the same age.

Fasting produced a significant decrease in the intestinal sucrase activity of male rats, and the maximum decrease from the normal value occurred after a fasting period of two to four days. When the fasting period was extended to twelve days, the enzyme activity remained relatively constant at the level observed at the end of the four-day period of fasting.

A 70% sucrose diet produced a highly significant increase in intestinal sucrase levels when fed ad libitum for 24 hours to rats previously fasted for three days. High dietary contents of fructose, or glucose plus

fructose in a 1:1 ratio, or maltose, produced statistically significant increases in sucrase activity, but diets which contained high levels of any one of glucose, xylose, or lactose caused no significant elevation in sucrase activity above the fasting levels of the enzyme. A carbohydrate-free-high casein diet which was used as the control diet and a carbohydrate free-high fat diet also produced no significant increases in the enzyme levels. The response of the intestinal sucrase of fasted rats to ingested sucrose probably is due to sucrase synthesis by the gastrointestinal tract in response to the appearance of the disaccharide. The effects produced by the other sugars in the diets offered to fasted rats must be considered in terms of the known in vitro properties of intestinal sucrase.

Male albino rats, not previously fasted as above, were maintained on a diet of ground Purina fox checkers for one week, and then they were fed a high sucrose diet for four weeks. These animals exhibited intestinal sucrase activities which were not significantly different from those of the control animals which subsisted on a diet of Purina fox checkers. However, a sucrose free-high casein diet fed to unfasted animals also previously fed fox checkers, caused a highly significant decrease in the levels of the enzyme, even after one day. The decrease in activity is again thought to be a response by the gastrointestinal tract to the amount of sucrose in the diet.

It would appear from the above observations that intestinal sucrase is one of the "adaptive" gastrointestinal enzymes which have a significant role in the hydrolysis of valuable dietary ingredients preparatory to the absorption of the simple products of digestion.

THE UNIVERSITY OF ALBERTA

STUDIES ON RAT INTESTINAL SUCRASE

A THESIS

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

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I. GENERAL INTRODUCTION

Sucrase action has been known since 1828 when Dumas and Boullay (24) showed that yeast fermentation of sucrose occurs only when one molecule of water is used in the reaction. Neuberg and Mandl credit Persoz with discovering the inverting action of yeast in 1833 (69), and the enzyme responsible for the phenomenon was isolated by alcoholic precipitation by Berthelot in 1860 (10). Berthelot gave the name "ferment inversif" to the enzyme. Today, invertase, derived from the original name coined by Berthelot, is synonymous with invertin, saccharase, and sucrase, although invertase is usually applied in a general sense, whereas sucrase is reserved for the enzymes which hydrolyze sucrose.

Kuhn (51) has differentiated two forms of invertase, and his classification has been substantiated by the recent discoveries of Bacon (5), Edelman (25), and White and Maher (105). According to Kuhn's differentiation, fructosidase (fructosaccharase, fructofuranosidase, β-h-fructosidase*, fructoinvertase) attacks the fructose side of the substrate molecule, whereas glucosidase (glucosaccharase, α-n-glucosidoinvertase**, α-glucopyranosidase, glucoinvertase) attacks the glucose side. Accordingly, both types can act on sucrose. Whether glucoinvertase is an enzymatic entity is a matter of dispute (86). This question will be discussed in detail later.

The intestinal sucrase of vertebrates appears to be α-glucopyranosidase, as is the invertase associated with invertebrates (69). β-h-fructosidases are found only in plants and bacteria. Both types of invertase are

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*The term β-h-fructosidase refers to the specificity of this enzyme for fructose with β-configuration and the 2,5-oxygen ring.

**The term α-n-glucosidoinvertase refers to the specificity of this enzyme for glucose with α-configuration and the 1,5-oxygen ring.

found in yeast (69), and the type of enzyme varies with the species in molds (69). Yeast invertase is the best known and most intensely investigated member of the β-h-fructosidase group.

The existence of sucrase in the animal intestine was reported in 1860 by Leube (54). Euler and Svanberg (26) and Röhmann (76) determined the approximate localization of the enzyme in intestinal tracts half a century later. Intestinal sucrase seems to have almost universal distribution among vertebrates, and it has been demonstrated in the intestinal tract of the foetus (62), the stillborn (47, 87), and adult humans (70); in the intestinal tract of pigeons (9) and rats (90); and in the gizzard of chickens (69), but not in the intestine of cattle (29) or newborn pigs (3).

The function of sucrase in the intestine of vertebrates in which it has been demonstrated has been assumed as the hydrolysis of sucrose and other substrates in the diet within the lumen of the intestine. It is well known that adult and growing rats have been maintained on diets containing sucrose as the only carbohydrate. Also, oral administration of sucrose is universally used by diabetics to counteract hypoglycemia. It was thought that the monosaccharide hydrolysis products (fructose and glucose from sucrose) were then absorbed from the intestinal lumen, probably by a phosphorylation process similar to that observed for non-hydrolytically derived monosaccharides (21, 39, 41, 90, 99). However, Fridhandler and Quastel (32) have shown that the major site of sucrose hydrolysis and the mechanism of absorption of the hydrolysis products is different from those assumed. Their investigations on isolated loops of guinea pig intestine showed that little hydrolysis of sucrose occurs in the intestinal lumen, but the rate of absorption of glucose and fructose is far greater than when a mixture of fructose and glucose, equal in concentration to that produced in the intestine lumen by hydrolysis of sucrose, is perfused through the intestine loop. Therefore, these workers

concluded that "extensive hydrolysis of sucrose does not occur in the lumen preparatory to the absorption of glucose and fructose, but most of the sucrose breakdown occurs after entry into the intestinal tissue has taken place."

The chemical nature, structure, and physical properties of the glucoinvertases are unknown. Very little is known about the kinetics and mode of action of glucoinvertases (including intestinal sucrase), and, in addition, as mentioned earlier, the identity of glucoinvertase is disputed. In connection with reaction kinetics, the pH optimum for glucoinvertases has been determined as 6-8, except for that of barley malt, in which case the optimum is 4.5-5.0 (69). Rat intestinal sucrase has apparently been investigated only by Tuba and Dickie (90), and, thus, very little information regarding this enzyme is available.

The mode of action of the investases is being investigated by a number of researchers, and their findings for both types of invertase illustrate the rapid progress being made in this field. It is now known (6, 7, 14) that the equation, Sucrose + $H_2O \longrightarrow$ Fructose + Glucose, does not correctly represent the state of the reaction at intermediate stages (4), although it represents the overall reaction catalyzed by various invertase preparations. Bacon (5) isolated two disaccharides other than sucrose, and three trisaccharides from incubation mixtures of yeast invertase and sucrose. Gross and collaborators (36, 37), have identified two of the trisaccharides as kestose, in which fructose is transferred to the 6-position of the fructose component of sucrose, and neo-kestose, in which the 6-position of the glucose component accepts fructose.

Fischer and Kohtès (28) have described a highly purified yeast invertase preparation which mediates the transfer of the fructose residue from sucrose, both to water and to acceptor carbohydrates, which confirms

the view that the same invertase functions both as a hydrolase and as a trans-fructosidase (5, 25, 28). In the presence of the enzyme, alcohols (e.g., methanol, glycerol, benzyl alcohol, glucose, sucrose, and other oligosaccharides) function as acceptors for the fructofuranose moiety, which may be donated by different fructosides possessing an unchanged and unsubstituted-fructofuranoside terminal group, e.g., sucrose, raffinose, and β-methylfructofuranoside. The enzyme readily hydrolyzes the variety of fructofuranosides which may be formed by the transfer process. The enzyme activity seems to be restricted to fructosides with an unsubstituted and unchanged β-fructofuranosidic terminal, and the cleavage occurs between the fructose carbon and the oxygen bridge (48).

Yeast and mold invertases show two distinct types of transfructosylase activity (5, 25) whereas honey (bee) invertase has transglucosylase activity (105). Presumably this transglucosylase activity is also characteristic of other glucosaccharases, including rat intestinal sucrase.

Glucoinvertase hydrolyzes sucrose and melizitose, but it is inert toward raffinose (35). Whether the enzyme is an enzymatic entity or is identical with animal maltase as Weidenhagen (101) maintains is a matter of dispute. Weidenhagen's argument is that maltase has inverting power at pH 7 after it has been purified to a degree which leaves it inactive towards sucrose at pH 4.5, the pH optimum for yeast invertase (69). Weidenhagen's view is not generally accepted, and several authors, especially Hofmann (42), have reported experiments to contradict his theories. Hofmann succeeded in separating maltase and invertase from Schizosarcharomyces octosporus Beijerinck, and he isolated a maltase which did not attack sucrose at any pH. In addition, maltase and sucrase preparations, which have only maltase and sucrase activity, respectively, have been prepared (69).

Weidenhagen (102) has also advanced the theory that all α- β-glucosidic linkages in naturally occurring oligosaccharides (maltose, sucrose, melizitose, turanose, and trehalose) and in synthetic α- D-glucosides, are split by the same enzyme, namely α- D-glucosidase. This theory has not been disproven, although it is well known that maltase present in blood serum, extracts of gastric mucosa, saliva, pancreatic extract, and mammary gland does not act on sucrose (69). However, the fact that maltases from different sources do not convert sucrose does not disprove the identity of maltase and glucopyranosidase since maltases vary in their range of substrate specificity (35). On the other hand, the disaccharide trehalose, which is an α-glucoside, resists the action of α-glucosidase and all other invertases (69). Also, the distinction between the various sucrases has been supported by the recent discoveries that yeast and mold invertases show two distinct types of transfructosylase activity (5, 25), whereas honey invertase has transglucosylase activity (105). The data seem to prove only the existence of two forms of sucrase.

The chemical nature of yeast invertase is in doubt, and its structure has only been postulated. However, indications of the reasons for the differences observed between invertases can be gained by consideration of the hypotheses advanced. Sumner and O'Kane (83) characterize invertase as a polysaccharide protein, and Adams and Hudson (2) call the enzyme a carbohydrate-protein, but Gortner and Dieu (33), basing their conclusions on polysaccharide and glucosamine analyses, and on previous electrophoresis studies of molecular weight, say that the active principle may be simply a"micro-protein." The nitrogen content of invertase preparations varies between 6.6 and 12.7 per cent, only part of which is amino nitrogen. These facts have caused some people (107, 108, 109) to doubt the protein nature of invertase. The polypeptide nature has not been disputed, however (69). The concept of the

invertase system being composed of an active group (agon) and a carrier (pheron) has been postulated and is related to the protein or polypeptide nature of the enzyme (50). The agon has been ascribed carbohydrate character, whereas the pheron is protein or a protein-like substance. Accompanying substances, such as phosphorus, may be due to impurities (69). The theory of the agon-pheron nature of invertase can explain the differences in behavior of various invertases, and also the existence of different forms of the enzyme, if the agon is assumed to combine to form active complexes with various pherons. Fructo- and glucoinvertases may contain the same agon but different pherons or accompanying substances. There is no reason why different invertases should not exist in the same kind of cell. Similarly, the theory explains the existence of atypical forms of the enzyme which attack substrates for both types of sucrase at an atypical pH, since the pheron is able to combine with a large variety of substances (69).

During their studies on the effects of carbohydrates on the levels of rat intestinal phosphatase, Tuba and Dickie (90), in a preliminary investigation, demonstrated that sucrase occurs in the rat intestine, and that lack of a statistically significant effect of sucrose on phosphatase was not due to lack of hydrolysis. Some of the reaction kinetics of intestinal sucrase were studied during this investigation. Since it had been shown that rat intestinal sucrase has a role in carbohydrate digestion and absorption, the present studies on rat intestinal sucrase were suggested to the author in order to elucidate more information about the action of the enzyme and about the various factors affecting its activity.

Most of the studies on intestinal sucrase have been conducted on dogs by the use of intestinal juice (succus entericus) obtained by means of intestinal fistulae. Tuba and Dickie (90) were the first to use intestinal homogenates for sucrase studies, and the use of these preparations has been continued in

this investigation which involves the development of a simple, but accurate, assay method, localization of the enzyme activity in the rat small intestine, study of the reaction kinetics and of the various dietary factors affecting the enzyme level, and a survey of sucrase activity in rat tissues.

II. THE ESTIMATION OF INTESTINAL SUCRASE ACTIVITY

A. METHODS AVAILABLE

Intestinal sucrase activity may be determined by incubating the enzyme preparation with sucrose under the proper conditions and then measuring the invert sugar produced.

The classical method of measuring the invert sugar produced has been the determination of either the change in optical rotation which occurs when sucrose is hydrolyzed, or the "time value" - the time required for the optical rotation of a sucrose solution to be brought to zero under standard conditions. However, measurement of optical rotation is accompanied by serious error if the operator of the saccharimeter has defective eyesight, and, furthermore, the method, although simple, is rather coarse, for it requires liberal use of both substrate and enzyme. Cloudy solutions, of course, cannot be used.

The reducing power of invert sugar may be determined by any of the common methods employed for glucose, and procedures of this type are quite popular, although methods employing the very specific Penicillium notatum glucose oxidase (notatin) can be used (40). The notatin method is very time-consuming in that notatin must first of all be prepared from cultures of Penicillium notatum. Many of the methods for determining the reducing power of invert sugar, requiring a large number of accurate measurements, are, likewise, time-consuming. In addition, many of the methods do not measure small quantities of reducing sugar. However, the 3,5-dinitrosalicylic acid reduction method described by Sumner and Howell (82) is quite accurate, involves few measurements, and is rapid.

B. METHOD ADOPTED

The method finally adopted for measuring intestinal sucrase activity is a modification of the method used by Tuba and Dickie (90), which in turn is a modification of the method used by Sumner and Howell (82) for determining yeast invertase.

1. Reagents

(i) Dinitrosalicylic Acid Reagent (81).

22 ml. of 10% NaOH are added to 10 grams of crystalline phenol, and the volume is made up to 100 ml.

6.9 ml. of the alkaline phenol solution are mixed with 6.9 grams of meta sodium bisulfite ($Na_2S_2O_5$). Then a solution containing 300 ml. of 4.5% NaOH, 255 grams of Rochelle salt ($KNaC_4H_4O_5.4H_2O$), and 880 ml. of 1% 3,5-dinitrosalicylic acid is added.

(ii) Glucose Standard Solutions.

D(+) glucose. C.P. anhydrous. Pfanstiehl Chemical Co.

(iii) 11% Sucrose in phosphate buffer

11 grams of sucrose are dissolved in 0.05 M phosphate buffer of pH 6.25 and made up to 100 ml.

Sucrose: Crystal. Analar. The British Drug Houses Ltd.

(iv) 0.05 M Phosphate Buffer, pH 6.25

8 parts 0.05 M KH_2PO_4 plus 2 parts 0.05 M Na_2HPO_4.

2. Development of the Method

In the method of Sumner and Howell (82) 5 ml. of 6.5% sucrose in acetate buffer are incubated with 1 ml. of saccharase solution at a pH of 4.5 at 20°C. for 5 minutes. The reaction is stopped at the end of 5 minutes by the addition of 5 ml. of 0.1 N NaOH. The invert sugar produced is determined by adding a 1 ml. aliquot of the reaction solution to 3 ml. of dinitrosalicylic acid reagent. The resulting solution is then mixed, heated in

boiling water for 5 minutes, cooled, and finally compared with a standard containing 1 mgm. of glucose per ml. (Sumner and Howell (82) state that there is no difference between the reducing values for invert sugar and for D-glucose.) Mgm. invert sugar x 11 = saccharase units per ml. enzyme solution.

Tuba and Dickie (90) demonstrated the existence of a relatively small amount of sucrase activity in the first 10 cm. section of the small intestine of the rat. The realization that further investigations of intestinal sucrase activity would require the measurement of small quantities of invert sugar prompted the search for a sensitive method of measuring invert sugar. Therefore, a spectrophotometric analysis of the colored glucose-dinitrosalicylic acid reagent reaction product was proposed to find the wavelength of maximum absorption, and also the wavelength of maximum sensitivity. These two wavelengths may or may not coincide. By measuring the per cent transmittance of the colored reaction product and then referring to a calibration curve, invert sugar and, therefore, sucrase activity can be determined.

The procedure adopted for use in measuring invert sugar was the following:

6 ml. of dinitrosalicylic acid reagent were added to 2 ml. of the solution containing invert sugar or glucose. The 3:1 ratio of reagent to sugar solution was adopted because this ratio was chosen for use in terminating and determining sucrose activity. (The dinitrosalicylic acid reagent itself is 0.246 N with respect to NaOH. Therefore, the addition of 5 ml. of 0.1 N NaOH to stop the enzyme action, used in Sumner's procedure, was eliminated in favor of adding the dinitrosalicylic acid reagent directly to the reaction solution. Thus, 6 ml. of dinitrosalicylic acid reagent would be added to 2 ml. of reaction solution.) The sugar solution and the dinitrosalicylic acid reagent were mixed, heated for 5 minutes in boiling water, and then cooled

for 3 minutes in cold, running tap water. The per cent transmittance of the colored solution was then determined by reading against a blank containing 1 ml. of 11% sucrose, 1 ml. of distilled water, and 6 ml. of dinitrosalicylic acid reagent in a Beckman Photoelectric Quartz Spectrophotometer.

In order to find the wavelengths of maximum light absorption and of maximum sensitivity, the sugar reagent was heated with glucose solutions of various concentrations (0.03, 0.07, 0.16, 0.25 and 0.45 mgm. of glucose per ml., respectively) and the per cent transmittance of each of the resulting colored solutions was determined by reading against a sucrose blank at wavelengths between 400 and 700 mμ. Maximum absorption was found to occur at 510 mμ. That this wavelength is also the wavelength of maximum sensitivity can be readily ascertained from Table I. Maximum sensitivity occurs at the wavelength where there is the greatest change in transmittance or optical density per unit change in concentration. Accordingly, 510 mμ was the wavelength chosen for all future measurements of per cent transmittance. The absorption curve for a solution containing 0.16 mgm. of glucose per ml. initially is shown in figure 1.

To prepare a calibration curve for determining invert sugar, 2 ml. of various concentrations of glucose in saturated benzoic acid were heated with the sugar reagent, and the per cent transmittance of the resulting solutions was determined by reading against a sucrose blank at 510 mμ. The concentrations of glucose used were the following: 0.025, 0.050, 0.060, 0.080, 0.10, 0.125, 0.150, 0.175 and 0.20 mgm. per ml. The results are presented in Table II. These data were used to prepare a standard calibration curve (fig. 2) by plotting per cent transmittance against glucose concentration on semi-log paper.

3. Evaluation of the Method

(i) pH Optimum, Time of Reaction, and Enzyme Concentrations

Preliminary experiments were carried out to determine the pH optimum, incubation time, and tissue homogenate concentration to be used in the assay for intestinal sucrase. A final sucrose concentration of 5.5%, similar to the sucrose concentration employed by Sumner and Howell (82), was used. This concentration is within the 5% to 10% sucrose range required for maximum action velocity by yeast invertase (68).

For these preliminary studies a tissue homogenate of the first 10 cm. section of the small intestine of an adult male albino rat was prepared. The rat was killed by decapitation, and the first 10 cm. of the small intestine from the pylorus was excised and placed in a beaker surrounded by chopped ice. The section was then cleaned by the removal of connective tissue, mesenteries, and blood vessels; washed with a stream of distilled water from a wash bottle; and blotted dry with filter paper. After it was weighed, the section was homogenized in cold distilled water in an ice-cooled Potter-Elvehjem glass homogenizer (74). The homogenate was made up to 25 ml. with cold distilled water in a volumetric flask, and suitable dilutions of this homogenate were tested for sucrase activity at a pH of 6.0, a sucrose concentration of 5.5% and a temperature of 37°C.

Results of these experiments indicated that a dilution of 1:4 of the original 25 ml. homogenate, a reaction time of 15 minutes, and a pH of 6.25 would be best for use in future experiments.

(ii) Accuracy of the Method

Replicate determinations were obtained which had transmittance values agreeing within 1% transmittance. The results of recovery tests are given in Table III. At glucose concentrations of 0.025, 0.05, 0.075, 0.10, 0.15, and 0.20 mgm. per ml. the percentage errors are +6.0, +12.0, +4.13, +3.00, +1.50,

and +2.00, respectively. The method tends to give high values rather than low ones. It was concluded from these data that the region of greatest accuracy was between 10 and 60% transmittance.

The variations noted fall within the range of the standard deviations of the means for the values determined for sucrase activity in intestinal sections, and do not affect the validity of these determinations.

4. Summary of the Method for Estimating Intestinal Sucrase Activity

1 ml. of intestinal homogenate in a 15 ml. test tube is warmed to 37°C. in a constant temperature water bath during a 10-minute equilibration period. 1 ml. of 11% sucrose in phosphate buffer of pH 6.25, similarly warmed to 37°C., is added to, and mixed with, the homogenate. After 15 minutes the reaction is stopped by adding exactly 6 ml. of dinitrosalicylic acid reagent from a burette.

Color is produced by heating the mixtures in boiling water for 5 minutes. After the solutions are cooled for 3 minutes in cold running tap water, the per cent transmittance of the colored solutions is determined at once* by reading against a sucrose blank at 510 mμ in the Beckman Photoelectric Quartz Spectrophotometer.

The number of mgm. of invert sugar produced is determined by reference to the standard calibration curve.

5. The Unit of Intestinal Sucrase Activity

Sumner and Howell (82) have suggested that the unit of saccharase activity be expressed simply as the number of mgm. of invert sugar produced in five minutes under standard conditions. Similarly, for intestinal sucrase the unit of activity has been chosen as equal to the number of mgm. of invert sugar produced in 15 minutes at 37°C. and pH 6.25 in 5.5% sucrose.

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*Sumner (81) states that there is no change in the value of the color intensity for at least one half-hour after the color has been developed.

Table I
The Relationship Between Absorbancy and Wavelength
for Colored Solutions Obtained by the
Dinitrosalicylic Acid Method for Glucose or Invert Sugar

Wavelength (mμ)	Absorbancy Glucose Concentration (Mgm. per ml. of Incubation Mix) 0.03	0.07	0.16	0.25	0.45
400		0.0505	0.1457	0.4290	0.7700
410		0.0434	0.1397	0.4290	0.7630
420		0.0246	0.1177	0.3950	0.7210
430		0.0044	0.0915	0.3540	0.6680
440		0.0000	0.1675	0.3010	0.6110
450		0.0000	0.1659	0.2970	0.6200
460		0.0088	0.2076	0.3420	0.6580
470		0.0374	0.2676	0.4200	0.7700
480	0.0000	0.0555	0.3170	0.4980	0.8860
490	0.0099	0.1024	0.4200	0.6530	1.0970
500	0.0339	0.1805	0.6580	1.0000	1.6480
510	0.0292	0.1805	0.7330	1.1550	2.0000+
520	0.0246	0.1367	0.6580	1.0340	2.0000+
540	0.0110	0.1024	0.4950	0.7960	1.6020
560	0.0044	0.0706	0.3820	0.5850	1.2040
580	0.0000	0.0362	0.2291	0.4060	0.8460
600		0.0315	0.1565	0.2696	0.5770
640		0.0022	0.0482	0.1079	0.2471
700		0.0000	0.0000	0.0212	0.0605

Table II
The Relationship Between Glucose Concentration and Transmittance

Glucose Concentration (Mgm. per Ml. of Incubation Mixture)	Equivalent Mgm. of Invert Sugar per Ml. of Homogenate	Per Cent Transmittance
0.025	0.050	95.50
0.050	0.100	84.25
0.060	0.120	74.00
0.080	0.160	56.50
0.100	0.200	42.50
0.125	0.250	29.25
0.150	0.300	20.50
0.175	0.350	14.00
0.200	0.400	10.25

Table III
The Recovery of Given Amounts of Glucose by the Dinitrosalicylic Acid Method for Estimating Intestinal Sucrase Activity

Number of Determinations	Glucose Concentration (Mgm. per Ml. of Incubation Mixture)	Mgm. of Glucose Added	Mgm. of Glucose Recovered	Per Cent Error of Method
2	0.025	0.050	0.053	+ 6.0
7	0.050	0.100	0.112	+12.0
7	0.075	0.150	0.158	+ 4.1
6	0.100	0.200	0.206	+ 3.0
5	0.150	0.300	0.306	+ 1.5
4	0.200	0.400	0.404	+ 2.0

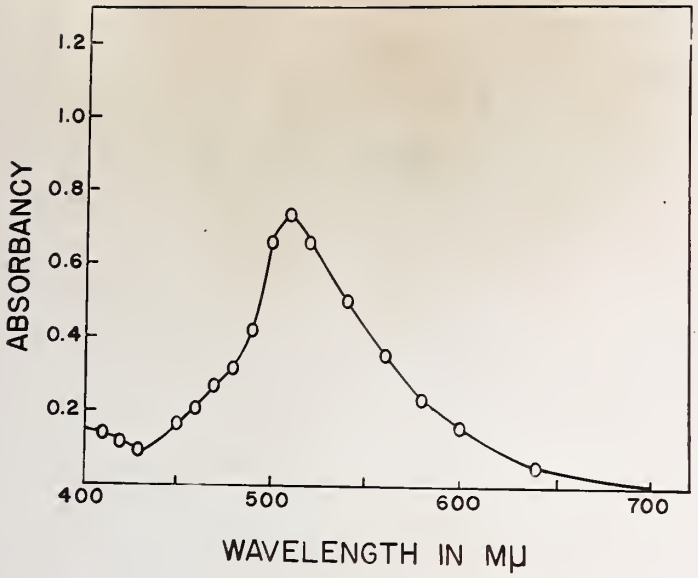

Fig. 1. The Relationship of Absorbancy to Wavelength in the Dinitrosalicylic Acid Determination of Glucose or Invert Sugar.

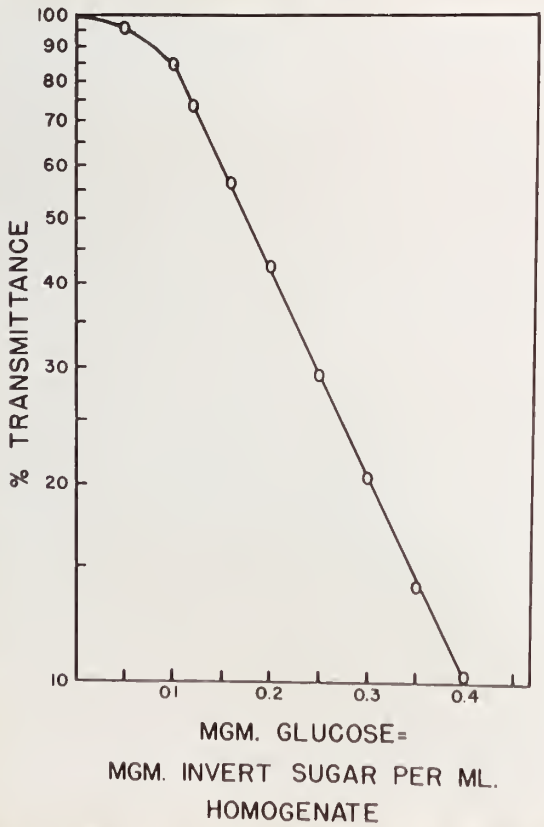

Fig. 2. The Calibration Curve for the Determination of Invert Sugar as Glucose by the Dinitrosalicylic Acid Method.

III. THE LOCALIZATION OF SUCRASE ACTIVITY IN THE SMALL INTESTINE OF THE RAT

1. INTRODUCTION

Nearly all experiments on intestinal sucrase in mammals have been done on the dog. The literature apparently contains no data regarding the localization of the enzyme in the intestinal tract of the rat. Most of the preparations examined have been either mucosal scrapings or intestinal juice, succus entericus, obtained from intestinal fistulae. Intestinal homogenates have been used in sucrase studies on rats only by Tuba and Dickie (90).

Leube reported an inverting action of intestinal juice in 1868 (54). Claude Bernard (1873) found activity in the intestinal mucosa as well as in the secretion (8), and Euler and Svanberg (1921) found that the concentration was greatest in the mucosa of the jejunum, less in the duodenum, and least in the ileum (26). However, Owles (70) found no relationship between intestinal levels and enzyme concentration in humans over the range of intestinal depths studied. Mitchell (61) states that sucrase and maltase are probably produced throughout the human small intestine.

Cajori (17), Koskowski (49), and Pierce, Nasset, and Murlin (72) have shown that whole intestinal juice digests more strongly than centrifuged juice, and the last two showed that digestion is still stronger if the juice is macerated with glass beads (31). Koskowski in 1926 showed that clear juice contained less sucrase than turbid or cellular juice (31). These findings indicate that intestinal homogenates should contain the greatest possible sucrase activity under experimental conditions, if sucrase is not removed during the process of cleaning the intestinal sections.

The small intestine of the rat consists of duodenum, jejunum, and ileum. The duodenum is in the form of a loop with descending, transverse,

and ascending limbs. The jejunum, which is thick-walled, begins where the duodenum turns posteriorly. The thin-walled ileum runs to the caecum.

The first part of the mammalian duodenum contains the duodenal or Brunner's glands embedded in its mucosa and sub-mucosa. The glands appear to contain a peptidase and an esterase, but amylase and enterokinase are the only significant enzymes (78). The mucosa which lines the rest of the small intestine consists of tubular glands (the crypts of Lieberkühn) and projecting villi. Both are covered by a single layer of epithelial cells with other cells interspersed between. The epithelial layer consists of simple cuboidal or cylindrical cells and goblet cells. Argentaffin cells (the flask-shaped cells of Kultschitzsky) scattered between the epithelial cells, are found mainly in the upper part of the intestine, while Paneth cells (cells with large acidophile granules) are present in the bases of the crypts of Liberkühn in some mammalian species (31). In man the cells of Paneth lie in the blind extremity of the glands of the ileum, but not in those of the duodenum (12). The question of whether the crypts secrete and the villi absorb, or whether they have a common function, has not been settled. Less is known definitely about the contribution of the Paneth and argentaffin cells to secretion (31). Because of their resemblance to the secreting cells of other digestive glands, the Paneth cells are believed to supply the enzymes of the intestinal juice (12). Some investigators have said that the cells of Kultschitzsky also possess this function (12).

It seems likely that the enzyme sucrase is not secreted from the cells into the intestinal juice. The amount of digestion by sucrase seems to parallel the cell content of the succus entericus, suggesting that the enzyme is derived from cast off epithelial cells rather than from the intestinal mucosa by secretion (31). However, Owles (70) claimed that he

definitely demonstrated that sucrase is secreted by the mucosa of the small intestine in humans.

In the following experiments, designed to determine the locale in which sucrase is concentrated in the rat small intestine, intestinal homogenates were used as the source of intestinal sucrase. Preliminary experiments have shown that about 6% of the total enzyme activity present in the intestinal sections is lost during the cleaning process; i.e., removal of feces and mucous material with distilled water. Thus, the sucrase activity associated with homogenates cannot be assumed to represent the in vivo activity or content of the enzyme, even though all other factors should be constant.

2. EXPERIMENTAL

The animals used in this experiment were adult male albino rats, which had been maintained on an ad libitum diet of Purina fox checkers and water.

The animals were killed by decapitation. Then the small intestine, from the pylorus to the caecum, was removed from each animal, and, beginning at the pyloric end, was cut into segments 10 cm. long. The sections were placed in beakers embedded in crushed ice, and those sections which could not be used immediately were frozen in the refrigerator until required.

At first all the sections were cleaned by removing mesenteries and blood vessels from the intestine proper, and then passing a stream of distilled water from a wash bottle through the section until all the fecal material was removed. The segments were then carefully blotted dry with blotting paper, and extreme care was taken to avoid expressing the fragile intestinal mucosa. In the lower sections of the intestine, where the feces were often firmly packed, much washing was required to remove this material.

It was found that sucrase activity was small or nonexistent in the lower sections washed in the manner described. Consequently, several more animals were sacrificed, and the excised intestinal sections were washed by a revised method. The mesenteries and blood vessels were removed, and then the sections were slit open lengthwise, and the fecal material was picked out with a scalpel. The sections were then washed with a stream of distilled water and blotted dry as before. This method proved to be more satisfactory for cleaning the lower intestinal sections (7th onward), although no significant difference was found between the two methods for the first six sections (Table IV).

The washed and blotted intestine in each case was weighed, and then homogenized in cold distilled water in a Potter-Elvehjem glass homogenizer (74) surrounded by crushed ice in an erlenmeyer flask.

The homogenate and washings were made up to volume in a 100 ml. volumetric flask and stored in the refrigerator at 5°C. Levels of sucrase activity were determined on one ml. of the homogenate by the dinitrosalicylic acid method previously described.

Blanks which contained homogenate and distilled water plus the dinitrosalicylic acid reagent gave average transmittance values ranging from 100 to 86.5%, corresponding to values of 0.0 to 0.096 mgm. of invert sugar. (It had previously been found that blanks which contained distilled water instead of sucrose gave the same percentage transmittance as ones which contained sucrose.) Non-substrate blanks for the first, second, and third intestinal segments were usually the ones which differed from the sucrose blank, and usually had the lowest per cent transmittance readings, although blanks for the other sections sometimes had lower readings. These blank values are thought to represent the amount of reducing substances present in the homogenate

preparations. Large divergence was found among the values for the blanks, due to the difficulty of making accurate measurements in the region of high transmission. The blank values (mgm. of invert sugar) were subtracted from the values obtained for the sucrase determinations in order to arrive at the absolute value for sucrase activity.

As mentioned in the introduction, a preliminary experiment was conducted to determine how much sucrase activity was removed from the intestinal sections by the washing process. For this study the third 10 cm. sections of the small intestines from five rats were washed by the first method described, and the washings for each section were collected separately and diluted to 100 ml. One ml. aliquots of the washings were then tested for sucrase activity, the time of incubation being one hour instead of fifteen minutes. The sucrase activities of the washings were then calculated for an incubation period of fifteen minutes in units per gram of wet tissue and compared with the activities obtained for the intestinal homogenates. The average amount of sucrase activity removed by the washing process was 5.94 per cent. Therefore, the sucrase activity associated with intestinal homogenates represents approximately 94% of the total sucrase activity.

The nitrogen content of the third 10 cm. sections of the small intestines of ten rats was determined in order to compare the method of basing results on the wet weight of the intestinal sections with that of expressing them in terms of nitrogen content. A 5 ml. sample of homogenate was used to perform a macro-Kjeldahl determination of nitrogen.

Statistical analyses were performed to test the significance of the experimental results. The "t" test, as outlined in "An Introduction to Applied Biometrics" by L. P. V. Johnson (45), was used to test for significant differences between means, the probability value, "P", being used as the

criterion of significance. The results were regarded as significant if the value of P was less than 0.05 and greater than 0.01, and highly significant if P was less than 0.01.

3. RESULTS

The results of the experiments are presented in Tables IV, V, and VI.

Sucrase activity was found to be concentrated in the first half of the small intestine. No significant difference was found between the sucrase activities of sections 2, 3, 4, and 5 in which the activity was the greatest. Activity was significantly lower in the first section and also in sections 6 to 11 where a gradual decrease in activity occurred.

The data in Table IV indicate that in the lower regions of the small intestine (section 7 onward), where sucrase activity is low and fecal material is usually densely packed, sucrase is almost completely removed by too vigorous washing during the washing process. However, for the upper sections, where the enzyme activity is high, and the intestinal contents are usually more fluid, no difference was found between the two washing methods investigated.

No relative difference was found between sucrase activity expressed in terms of the wet weight of the tissue and sucrase activity expressed in terms of the nitrogen content. Thus, the method of expressing activity in terms of nitrogen content seems comparable to expressing it in terms of tissue wet weight. Intestinal nitrogen is not all derived from the protein of the intestinal tissue, and all the protein of the tissue is not enzymic, but intestinal nitrogen and intestinal sucrase are directly related since both are related to tissue protein.

Table IV

A Comparison Between Two Methods Used for Washing Intestinal Sections

Method I: Fecal Material Removed by Washing Only

Method II: Fecal Material Removed with Scalpel and by Washing

Section Number	Method I		Method II		Test of Significant Difference Between Methods. Value of "P"
	Number of Animals	Sucrase Activity (Units per gram of wet tissue)	Number of Animals	Sucrase Activity (Units per gram of wet tissue)	
1	6	29.6 ± 2.23*	4	33.7 ± 3.59*	0.05
2	6	47.3 ± 3.07	4	46.2 ± 2.35	0.05
3	6	42.0 ± 2.53	4	46.1 ± 4.08	0.05
4	6	43.2 ± 2.59	4	43.3 ± 3.28	0.05
5	6	39.9 ± 2.88	4	42.8 ± 1.10	0.05
6	6	24.2 ± 2.00	4	29.3 ± 3.89	0.05
7	6	1.88 ± 1.86	4	19.3 ± 1.49	0.01
8	6	0.74 ± 0.74	4	16.3 ± 3.40	0.01
9	6	None	4	9.38 ± 1.16	0.01
10	6	None	4	9.66 ± 1.72	0.01
11	6	None	4	5.16 ± 1.89	0.02

*Standard error of the mean.

Table V
The Localization of Sucrase Activity in the Small Intestine of the Adult Male Albino Rat

Number of Animals	Section Number	Sucrase Activity (Units per gram of wet tissue)
10	1	31.1 ± 1.91*
10	2	46.9 ± 1.99
10	3	43.7 ± 2.22
10	4	43.2 ± 1.71
10	5	41.1 ± 1.78
10	6	26.2 ± 2.02
4	7	19.3 ± 1.49
4	8	16.3 ± 3.40
4	9	9.38 ± 1.16
4	10	9.66 ± 1.72
4	11	5.16 ± 1.89

*Standard error of the mean.

Table VI
The Relationship Between Sucrase Activity, the Wet Weight of Intestinal Sections, and the Nitrogen Content of the Tissue

Animal Number	Wet Weight of Section in Gm.	Nitrogen Content of Section in Mgm.	Mgm. N per 100 Gm. Wet Tissue	Sucrase Activity		
				Units per Intestinal Section	Units per Gm. of Wet Tissue	Units per 100 Mgm. N
1	0.44	12.38	2,820	15.6	35.4	126
2	0.69	17.98	2,610	26.8	38.8	149
3	0.65	19.02	2,930	34.8	53.6	183
4	0.78	20.24	2,600	31.0	39.8	153
5	0.71	16.28	2,300	28.0	39.4	172
6	0.63	15.36	2,440	21.6	35.8	141
7	0.55	16.20	2,940	24.5	44.6	151
8	0.69	19.12	2,780	38.1	55.2	198
9	0.70	18.78	2,680	32.2	46.0	172
10	0.76	19.14	2,520	36.4	47.9	190

4. DISCUSSION

Although no attempt was made to separate the rat small intestine into duodenum, jejunum, and ileum, a rough comparison can be made between the results of these experiments and the results of Euler and Svanberg (26) who found that the concentration of sucrase was greatest in the mucosa of the jejunum of dogs, less in the duodenum, and least in the ileum. The first 10 cm. section of the intestine, which includes almost all of the duodenum, contained less sucrase activity than the second, third, fourth and fifth sections which include approximately 2 cm. of the duodenum and all of the jejunum. The rest of the sections, constituting the ileum, contained the least activity. Therefore, these results agree with those of Euler and Svanberg. These findings are also compatible with the general conclusion that enzyme activity is concentrated in the upper portion of the small intestine and absorption in the lower sections (79).

Dickie found that 6.90 mgm. of invert sugar were produced per gram of wet tissue by sucrase in homogenates of the first 10 cm. section of the rat small intestine after a 1 hour incubation of homogenate and substrate at 37°C. and a pH of 6.0 (22, 90). The results of the present investigation indicate that sucrase activity of at least sixteen times this value exists in the first section. The possible reasons for this discrepancy between values are that in Dickie's method (a) a factor of 11 was apparently not taken into consideration in the calculations (22), (b) the pH was 6.0 instead of 6.25 ± 0.15, (c) most of the per cent transmittance readings were in the 80 to 100 per cent range where the error of measurement is quite large (22), (d) the wavelength used was 560 mμ instead of 510 mμ, and (e) the initial substrate concentration was $5/6 \times 6.5 = 5.4\%$ instead of the 5.5% concentration used in the present experiments. The substrate concentrations are both within

the range of concentrations at which the enzyme is saturated (68). Therefore, this small difference in sucrose concentrations should cause no difference in sucrase activity. The differences in pH, wavelength, and substrate concentration noted probably account for little of the discrepancy between the values for sucrase activity. The major factors involved are those of calculation and measurement of percentage transmittance. It must be pointed out that Dickie was concerned chiefly with proving the existence of the enzyme in the intestine.

Since most sucrase activity was found in intestinal sections 2, 3, 4, and 5, the third section was chosen for all the following studies.

5. SUMMARY

1. Rat intestinal sucrase activity is greatest in the second, third, fourth, and fifth 10 cm. sections of the small intestine, less in the first section, and least in the sixth and following sections.

2. Under the experimental conditions employed, the sucrase activity in the third 10 cm. section of the small intestine of normal adult male rats was found to be 43.7 ± 2.22 units per gram of wet tissue.

3. The demonstrable sucrase activity in the lower sections of the small intestine is decreased by too vigorous washing during the washing procedure.

IV. THE KINETICS OF THE ACTION OF RAT INTESTINAL SUCRASE

1. INTRODUCTION

The subject of the kinetics of enzyme reactions deals with the velocity or rate at which the reactions occur and the factors which influence this rate. A knowledge of enzyme reaction kinetics is necessary in order to develop an accurate method for estimating enzyme activity, and it also provides clues to the mode of enzyme action.

The factors which will be considered in the study of the kinetics of rat intestinal sucrase are (1) pH, (2) substrate concentration, (3) temperature, (4) time of reaction, (5) enzyme concentration, and (6) storage time.

The literature presents little information regarding the kinetics of glucoinvertase and apparently none for the kinetics of rat intestinal sucrase. However, a great deal of information regarding yeast invertase kinetics has been accumulated.

Glucoinvertases are most active at pH 6-8 with the exception of the invertase of barley malt (pH 4.5 to 5.0) (69). Intestinal sucrase, it is reported, has an optimum pH of 6-8 (69). Masuta Mori (64) reported that sucrase, amylase, and maltase from the intestinal mucosa of the rabbit have their highest activity at pH 5-7. Invertases of different origins show optimum values ranging from pH 4.2 to 7.0, and shifts have been observed (69). Yeast invertase activity falls off rather rapidly on the alkaline side and more slowly on the acid side (40).

The range of substrate concentration promoting maximum reaction velocity for α-glucoinvertase, and the Km values, seem to be lacking in the literature. For comparison, however, Km values for yeast invertase have been

reported as 0.0161 M (55), 0.016 M (52), and 0.028 M (66). Variations in the value of Km occur with the source of the yeast. Nelson and Vosburgh (68) have shown that sucrase attains its maximum velocity in solutions of five to ten per cent sucrose. Sumner and Howell (82) employed a solution having a final sucrose concentration of 5.4% in estimating yeast invertase activity. These sucrose concentrations might, by chance, also be optimal for rat intestinal sucrase.

The activation energy, μ, for yeast invertase is given as 11,000 calories per mole at temperatures above -2°C. and 60,000 calories per mole at lower temperatures (77). An activation energy of 11,000 calories per mole corresponds to a temperature coefficient, Q_{10}, of 1.61, the value reported by Nelson and Bloomfield (67). Similar data are lacking for α-glucoinvertases.

When an enzyme is saturated with substrate, which would occur in a substrate concentration in which the reaction velocity is maximal, the reaction theoretically should be zero order. The reaction rate should then be constant over a period of time and should not be influenced by the concentration of the reactants. In solutions of 5 to 10% sucrose, the invertase-catalyzed hydrolysis of sucrose follows a zero order reaction (68). The same phenomenon should be exhibited by the rat intestinal sucrase-catalyzed reaction.

It has been shown that the amount of sucrose hydrolyzed is directly proportional to the concentration of highly purified invertase over a wide range of concentrations (60). When intestinal homogenates are used, however, this relationship may not be true over as wide a range, because the enzyme preparation is not in a pure form and is subject to the influences of any inhibitors present, and also to those of innumerable unknown factors.

2. EXPERIMENTAL

Intestinal homogenates of the third 10 cm. section of the small

intestine of the rat were used for all investigations of the kinetics of rat intestinal sucrase. Normal male albino rats, maintained on an ad libitum diet of Purina fox checkers and water, were killed by decapitation. The third 10 cm. section of the small intestine, measured from the pylorus, was excised and placed in a beaker surrounded by crushed ice. After this step was completed, the section was cleaned. The adhering pieces of mesenteries, fat, and connective tissue were dissected away. Then the section was split longitudinally, and the fecal material was picked out with a scalpel. Finally, the section was washed with a stream of distilled water from a wash bottle. The section was then weighed to within 0.01 gram and homogenized in an ice-cooled Potter Elvehjem homogenizer (74). The homogenate in each instance was made up to a 100 ml. volume in a volumetric flask and stored in the refrigerator at 5°C.

One ml. aliquots of the homogenates were used to determine sucrase activity by the dinitrosalicylic acid reduction method. The method of studying the kinetics of rat intestinal sucrase action is discussed separately for each factor considered. All values are the means of duplicate determinations.

(1) The Effect of pH on the Rate of Intestinal Sucrase Action

The effect of pH, or hydrogen ion concentration, on the velocity of intestinal sucrase action was studied by allowing the enzyme to act under conditions of varying pH. Buffers ranging from pH 2.5 to pH 10 were prepared, and then buffered substrate solutions of 11% sucrose were made from the buffers. In order to cover the pH range and use phosphate as the buffer in the region of optimum activity, three buffer systems of the same molarity were used. Phosphate buffer was chosen because it is found naturally as a buffer system in animal tissues.

The composition of the buffers, adapted from tables in "Outlines of Biochemistry" by Gortner, Gortner, and Gortner (34) and in "Manometric Techniques and Tissue Metabolism," by Umbreit, Burris, and Stauffer (97), are as follows:

A. 0.05 M Potassium phthalate buffers, pH 2.5 to pH 5.0

pH 2.5: - (50.0 ml. 0.2 M $KH_2C_8H_4O_4$ + 37.0 mg. 0.2 M HCl) diluted to 200 ml.

pH 3.0: - (50.0 ml. 0.2 M $KHC_8H_4O_4$ + 21.5 ml. 0.2 M HCl) diluted to 200 ml.

pH 4.0: - 50 ml. 0.2 M $KHC_8H_4O_4$ diluted to 200 ml.

pH 4.5: - (50 ml. 0.2 M $KHC_8H_4O_4$ + 9.00 ml. 0.2 M NaOH) diluted to 200 ml.

pH 5.0: - (50 ml. 0.2 M $KHC_8H_4O_4$ + 22.5 ml. 0.2 M NaOH) diluted to 200 ml.

B. 0.05 M Phosphate buffers, pH 5.6 to pH 8.0

pH 5.6: - 9.55 parts 0.05 M KH_2PO_4 + 0.45 parts 0.05 M Na_2HPO_4

pH 5.8: - 9.25 parts 0.05 M KH_2PO_4 + 0.75 parts 0.05 M Na_2HPO_4

pH 6.0: - 9.00 parts 0.05 M KH_2PO_4 + 1.00 part 0.05 M Na_2HPO_4

pH 6.1: - 8.50 parts 0.05 M KH_2PO_4 + 1.50 parts 0.05 M Na_2HPO_4

pH 6.2: - 8.20 parts 0.05 M KH_2PO_4 + 1.80 parts 0.05 M Na_2HPO_4

pH 6.25: -8.00 parts 0.05 M KH_2PO_4 + 2.00 parts 0.05 M Na_2HPO_4

pH 6.30: - 7.80 parts 0.05 M KH_2PO_4 + 2.20 parts 0.05 M Na_2HPO_4

pH 6.40: - 7.50 parts 0.05 M KH_2PO_4 + 2.50 parts 0.05 M Na_2HPO_4

pH 6.50: - 7.00 parts 0.05 M KH_2PO_4 + 3.00 parts 0.05 M Na_2HPO_4

pH 6.80: - 4.50 parts 0.05 M KH_2PO_4 + 5.50 parts 0.05 M Na_2HPO_4

pH 7.0: - 4.00 parts 0.05 M KH_2PO_4 + 6.00 parts 0.05 M Na_2HPO_4

pH 8.0: - 0.60 parts 0.05 M KH_2PO_4 + 9.40 parts 0.05 M Na_2HPO_4

C. 0.05 M Borate-KCl buffers, pH 9 and 10

pH 9.0: - (50 ml. borate-KCl (12.4048 g. boric acid + 14.912 g. KCl per litre) + 20 ml. 0.02 M NaOH) diluted to 200 ml.

pH 10.0: - (50 ml. borate-KCl + 43 ml. 0.2 M NaOH) diluted to 200 ml.

The pH of the reaction medium, composed of 1 ml. of buffered sucrose added to 1 ml. of intestinal homogenate, was determined, before and after incubation, with a Beckman pH meter. The pH before and after the reaction in no case differed by more than 0.13 pH unit. Intestinal sucrase activity, determined at the different pH's, is plotted vs. the average pH during the reaction in figure 3. Data for the plot are given in Table VII.

(2) The Effect of Substrate Concentration on the Rate of Intestinal Sucrase Action. Determination of Km.

The effect of substrate concentration on intestinal sucrase action was studied by measuring the reaction velocity under the usual conditions in concentrations of sucrose ranging from 0.01 M (0.34%) to 0.6 M (20.5%). The substrate concentration was plotted against reaction velocity (mgm. of invert sugar produced in 15 minutes) (figure 4) to illustrate the region of maximum velocity or region of enzyme saturation.

The postulated reaction between an enzyme and its substrate is that the enzyme (E) first combines with the substrate to form a compound (ES) by a reversible reaction

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

where k_1 is the rate constant for ES formation, and k_2 is the rate constant for the dissociation of ES to E + S. Following ES formation, S is converted into the reaction products (P) leaving E available for further ES formation. The overall reaction is

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E$$

where k_3 is the rate constant for the conversion of ES to P + E.

Assuming that the reaction $E + S \longrightarrow ES$ is a reversible process, then, according to the Mass Law, the following equation can be written:

$$Km = \frac{[(E) - (ES)]\ (S)}{(ES)} \qquad (1)$$

Km = the dissociation constant for ES

where (E) = total concentration of enzyme

(S) = total concentration of substrate chosen to be much greater than (E)

(ES) = concentration of enzyme-substrate complex

(E) - (ES) = concentration of free enzyme.

Rearranging equation (1) gives

$$(ES) = \frac{(E)\ (S)}{Km + (S)} \qquad (2)$$

If the velocity constant for the decomposition of ES is k_3, and the measured velocity is v, then $v = k_3(ES)$ and

$$v = \frac{k3(E)\ (S)}{Km + (S)} \qquad (3)$$

The maximal velocity, V_{max}, is attained when the concentration of ES is maximal, i.e. (E) = (ES). Then,

$$V_{max} = k_3(ES) = k_3(E)$$

By substituting V_{max} for k (E) in (3) the Michaelis-Menten equation is obtained.

$$v = \frac{V_{max}(S)}{Km + (S)} \qquad (4)$$

If the reciprocal of equation (4) is taken the following linear equation is obtained:

$$\frac{1}{v} = \frac{Km}{V_{max}(S)} + \frac{1}{V_{max}} \qquad (5)$$

If equation (5) is multiplied through by (S) we obtain

$$\frac{(S)}{v} = \frac{Km}{V_{max}} + \frac{(S)}{V_{max}} \qquad (6)$$

When $\frac{(S)}{v}$ is plotted vs. (S) the ordinate intercept is $\frac{Km}{V_{max}}$, and the constant slope is $\frac{1}{V_{max}}$.

If various concentrations of substrate are incubated with a standard amount of enzyme and the experimental values of (S) and $\frac{(S)}{v}$ are plotted against

each other, the slope and ordinate intercept of the resulting straight line can be determined. From these data Km can be calculated.

However, an algebraic determination of Km is more accurate. If for a series of $\underline{n}$ determinations $\sum(S)$, $\sum\frac{1}{(S)}$, $\sum\frac{(S)}{v}$, and $\sum\frac{1}{v}$ are determined and these values are then substituted in the formulas

$$\sum\frac{1}{v} = \frac{n}{V_{max}} + \sum\frac{1}{(S)} \cdot \frac{Km}{V_{max}}$$

$$\sum\frac{(S)}{v} = n\frac{Km}{V_{max}} + \sum(S)\cdot\frac{1}{V_{max}}$$

solving of the two simultaneous equations obtained for V_{max} yields the value of Km. Knowing Km, we can calculate V_{max}, and v and (S) can be determined by substituting in equation (4). Comparison of the calculated values of v with the experimental values affords a check on the accuracy of the experimental data.

The significance of Km is that it is the substrate concentration at half maximum velocity. It has the dimensions of concentration, e.g., molarity, and is constant for an enzyme under rigidly standard conditions. The experimentally determined value of Km represents the dissociation constant of ES only if the velocity of the formation of ES is much greater than the rate of its decomposition to products and E (16).

The rate of formation of ES may be denoted as proportional to the concentrations of substrate and free enzyme by the following differential equation:

$$\frac{d\,(ES)}{dt} = k_1\left[(E) - (ES)\right]\ (S)$$

The rate of decomposition of ES is represented by

$$-\frac{d(ES)}{dt} = -k_2(ES) - k_3(ES)$$

The overall change in concentration, therefore, is

$$\frac{d\,(ES)}{dt} = k_1\left[(E) - (ES)\right]\ (S) - k_2(ES) - k_3(ES)$$

As long as the rate of reaction (v) is constant, (ES) is constant and the term $\frac{d(ES)}{dt} = 0$. Then

$$(ES) = \frac{k_1(E)\ (S)}{k_1(S) + k_2 + k_3} = \frac{(E)\ (S)}{(S) + (k_2+k_3)/k_1}$$

If $(k_2 + k_3)/k_1$ is set equal to Km, the expression is the same as that derived by Michaelis. Thus, the Km value calculated from experimental data only approaches the dissociation constant, k_2/k_1, when k_2 is much greater than k_3.

From the values of v obtained experimentally the values of S/v were determined and plotted against the substrate concentration, S. Then the values determined for the ordinate intercept and the slope of the straight line were used to calculate the Km value. The Km value was also calculated algebraically.

Data for the determination of Km are given in Table VIII (b). The plot of S/v vs. S is shown in figure 5.

(3) The Effect of Temperature on the Rate of Intestinal Sucrase Action

An increase in temperature generally results in an increase in the rate of a chemical reaction. All enzyme-catalyzed reactions are known to increase in velocity with increased temperature until an optimum temperature is reached. A rise of 10°C. multiplies the rate by about 1.33 to 3.5 which represents the range of the Q_{10} value.

Arrhenius in 1889 proposed the equation

$$\frac{d \ln k}{d T} = \frac{A}{RT^2}$$ to describe the effect of temperature on reaction rate, where k = reaction velocity constant, T = absolute temperature, R = the gas constant (1.987 calories per degree per mole), and A = a constant. Integration of Arrhenius's equation between two temperatures, T_1 and T_2, corresponding to velocity constants k_1 and k_2 respectively, gives the equation

$$\log\left(\frac{k_2}{k_1}\right) = \frac{A}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

By plotting log k vs. $\frac{1}{T}$ a straight line of slope $\frac{A}{2.303R} = \frac{\mu}{4.58}$ is obtained, where A = μ, the activation energy. μ can be calculated from the value obtained for the slope.

According to the collision theory of chemical action, A, or μ, is the minimum energy that reacting molecules must possess before they will react. Eyring (27) proposed that the reactants in a chemical reaction, in order to form the final products, must form an activated complex whose energy level is higher than that of the reactants. The reactants are assumed to be in equilibrium with the activated complex, and if the equilibrium constant for the reaction is denoted by K, the change in free energy, ΔF, occurring when the activated complex is formed, is denoted by

$$\Delta F = -RT \ln K = \Delta H - T\Delta S$$

where R = gas constant, T = absolute temperature, ΔH = change in heat content and ΔS = change in entropy. A of the Arrhenius equation is directly related to ΔH.

The role of an enzyme is to cause the formation of an activated complex at a lower energy level than the activated complex formed in the absence of the enzyme. The enzyme acts to lower the energy barrier which a substrate, or substrates, must overcome before being converted into the final products.

In order to study the effect of temperature on the rate of intestinal sucrase action and to determine the activation energy, μ, the reaction tubes were incubated in a water bath thermostatically controlled to within 0.01° of the temperature. Incubations at temperatures below room temperature were conducted in the water bath inside a refrigerator kept at a temperature near 5°C.

Reaction velocities were determined at various temperatures between 5° and 40°C., and $\log_{10}$ reaction rate was plotted vs $\frac{1}{T} \times 10^4$ (figure 6). The activation energy, μ, was calculated from the slope of the straight line. The data used are given in Table IX.

(4) The Effect of Time on the Rate of Intestinal Sucrase Action

In the hydrolysis of sucrose

$$\text{sucrose} + H_2O \longrightarrow \text{fructose} + \text{glucose}$$

the mechanism is probably bimolecular, but the order of reaction is first (at low sucrose concentrations) since water concentration is essentially constant. For almost any enzyme reaction, if the substrate concentration is initially very high, i.e. the enzyme is saturated with substrate, the rate will at first describe a zero-order course and later decrease with increased time of reaction.

A zero-order reaction is described by the differential equation

$-\frac{dS}{dt} = k'$ where S = concentration of substrate, t = time of reaction, k = rate constant for the reaction and $-\frac{dS}{dt}$ = velocity, V, of the reaction. Integration of the equation yields

$k' = x/t$ where x = amount of substrate changed in time, t. The rate is constant over a period of time and is proportional to the enzyme concentration; $k' = KE = x/t$.

Zero-order kinetics were tested by allowing the reactions to continue under the usual conditions for lengths of time ranging from 5 to 30 minutes for the 100 ml. homogenate, and from 15 to 120 minutes for a homogenate diluted 1:5. The enzyme activity, determined at the end of each incubation period, was plotted vs. reaction time (figure 7). Data for this experiment are presented in Table X.

(5) The Effect of Enzyme Concentration on the Rate of Intestinal Sucrase Action

With highly purified enzymes the initial rate of the catalyzed reaction is directly proportional to the enzyme concentration over a fairly wide range (59).

The rate of a zero-order reaction is proportional to the enzyme concentration. Therefore, a halving of the enzyme concentration should result in a halving of the rate of enzyme action if time and the velocity constant remain constant. Since a pure enzyme is not being dealt with here, enzyme concentration has been assumed proportional to the weight of intestinal tissue and, therefore, to homogenate dilution.

An intestinal homogenate of 100 ml. was further diluted to produce the following dilutions:

1:1.25, 1:1.50, 1:2.0, 1:2.5, 1:3.0, 1:4.0, 1:5.0 and 1:6.0.

One ml. of each of these homogenates was incubated with buffered substrate and the sucrase activity was determined. The data of Table XI are plotted in figure 8.

(6) The Effect of the Storage Time on the Rate of Intestinal Sucrase Action; Enzyme Stability.

The stability of intestinal sucrase in intestinal homogenates stored in the refrigerator at 5°C. was tested at various times up to thirty-eight days after preparation. The sucrase activity was determined in the usual manner, and the activities at the end of the various time intervals were compared with the initial activity.

3. RESULTS

Results of the experiments on kinetics are presented in Tables VII to XI (pp. 39-44) and figures 3 to 8 (pp. 45-48).

Table VII
The Effect of pH on the
Rate of Intestinal Sucrase Action

Average pH During Reaction	Rate of Intestinal Sucrase Action, or Sucrase Activity (Mgm. invert sugar formed in 15 min.)
2.50	0.000
3.14	0.030
4.01	0.076
4.51	0.104
5.11	0.270
5.67	0.326
5.92	0.330
6.26	0.342
6.29	0.344
6.47	0.338
6.83	0.334
7.00	0.326
7.43	0.250
7.90	0.166
8.67	0.110
9.41	0.030

Table VIII
The Effect of Substrate Concentration
on the Rate of Intestinal Sucrase Action

(a) Determination of Maximal Sucrose Concentration

S (Molar)	v (Mgm. Invert Sugar formed in 15 min.)	Rate of Intestinal Sucrase Action, or Sucrase Activity (Mgm. Invert Sugar formed in 15 min.)
0.02	0.182	0.182
0.03	0.258	0.258
0.04	0.276	0.276
0.05	0.290	0.290
0.06	0.306	0.306
0.08	0.326	0.326
0.10	0.326	0.326
0.11	0.328	0.328
0.12	0.328	0.328
0.16	0.328	0.328
0.20	0.326	0.326
0.25	0.334	0.334
0.30	0.332	0.332
0.40	0.332	0.332
0.50	0.334	0.334
0.55	0.316	0.316
0.60	0.314	0.314

Table VIII (continued)

(b) Determination of the Km Value for Intestinal Sucrase

S (Molar)	$\frac{1}{S}$	v (Mgm. invert sugar formed in 15 min.)	$\frac{1}{v}$	$\frac{S}{v}$
0.01	100.00	0.168	5.952	0.059
0.02	50.00	0.182	5.495	0.110
0.03	33.33	0.258	3.876	0.118
0.04	25.00	0.276	3.623	0.145
0.05	20.00	0.290	3.448	0.173
0.06	16.67	0.306	3.268	0.196
0.07	14.29	0.304	3.289	0.231
0.08	12.50	0.326	3.067	0.245
0.10	10.00	0.326	3.067	0.307
0.11	9.09	0.328	3.049	0.336
0.12	8.33	0.328	3.049	0.366
0.14	7.14	0.316	3.165	0.444
0.16	6.25	0.328	3.047	0.489
0.18	5.56	0.316	3.964	0.570
0.20	5.00	0.326	3.067	0.613
0.25	4.00	0.334	2.194	0.750
0.30	3.33	0.332	3.012	0.904
0.40	2.50	0.332	3.012	1.205
0.45	2.22	0.334	2.994	1.345
0.50	2.00	0.334	2.994	1.495

$\Sigma S = 3.27, \Sigma \frac{1}{S} = 337.21$ $\Sigma \frac{1}{v} = 69.635$ $\Sigma \frac{S}{v} = 10.098$

$n = 20$ $V_{max} = 0.349$ $Km = 0.0128$

Table IX
The Effect of Temperature on the
Rate of Intestinal Sucrase Action

Temperature °C.	Temperature °A	$\frac{1}{T} \times 10^4$	Rate of Intestinal Sucrase Action (Mgm. invert sugar formed in 15 min.)	Log_{10} Reaction Rate
5	278.18	35.1	0.056	-1.252
10	283.18	35.3	0.076	-1.110
15	288.18	34.7	0.100	-1.000
20	293.18	34.1	0.126	-0.841
25	298.18	33.5	0.172	-0.765
30	303.18	33.0	0.240	-0.620
37	310.18	32.2	0.364	-0.439
40	313.18	31.9	0.404	-0.394

Table X
The Effect of Time on the
Rate of Intestinal Sucrase Action

Time (t) (Minutes)	Mgm. Invert Sugar (x) per ml. Homogenate	Rate of Intestinal Sucrase Action = Sucrase Activity (Mgm. invert sugar formed in 15 min.)	$K' = x/t$ (Mgm. invert sugar formed per minute)
(a) 100 ml. - Intestinal Homogenate			
0	0.000	0.000	
5	0.056	0.056	0.0115
8	0.108	0.108	0.0135
10	0.128	0.128	0.0128
12	0.160	0.160	0.0133
15	0.200	0.200	0.0133
18	0.236	0.236	0.0131
20	0.276	0.276	0.0138
25	0.335	0.335	0.0134
30	0.400	0.400	0.0133
(b) Homogenate of (a) diluted 1:5			
0	0.000	0.000	
15	0.032	0.032	0.00213
30	0.084	0.084	0.00270
45	0.012	0.122	0.00271
60	0.160	0.160	0.00267
75	0.200	0.200	0.00267
90	0.215	0.215	0.00238
105	0.240	0.240	0.00228
120	0.260	0.260	0.00217

Table XI
The Effect of Enzyme Concentration
on the Rate of Intestinal Sucrase Action

Homogenate Dilution	Relative Concentration of Enzyme	Mgm. Invert Sugar per ml. Homogenate	Rate of Intestinal Sucrase Action = Sucrase Activity (Mgm. invert sugar formed in 15 min.)
1:1.00	1.00	0.356	0.356
1:1.25	0.80	0.280	0.280
1:1.50	0.67	0.234	0.234
1:2.00	0.50	0.170	0.170
1:3.00	0.33	0.126	0.126
1:4.00	0.25	0.102	0.102
1:5.00	0.20	0.070	0.070
1:6.00	0.17	0.060	0.060

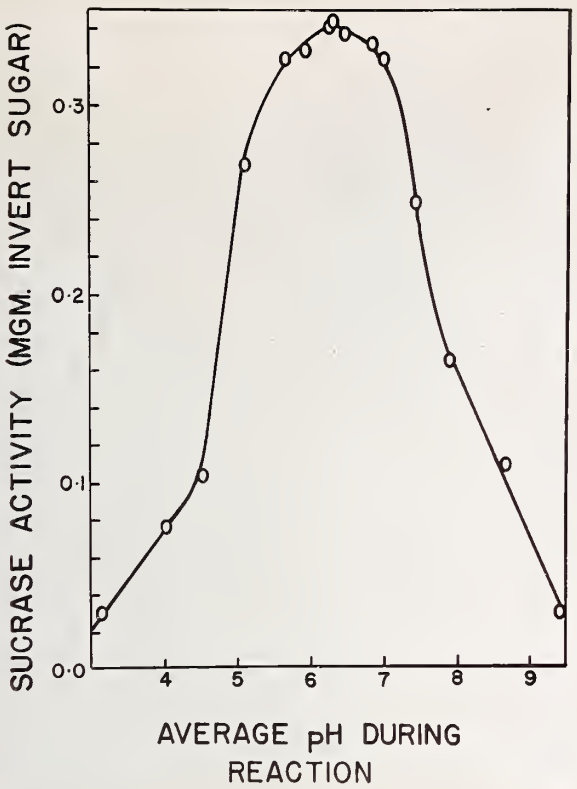

Fig. 3. The Effect of pH on the Rate of Intestinal Sucrase Action.

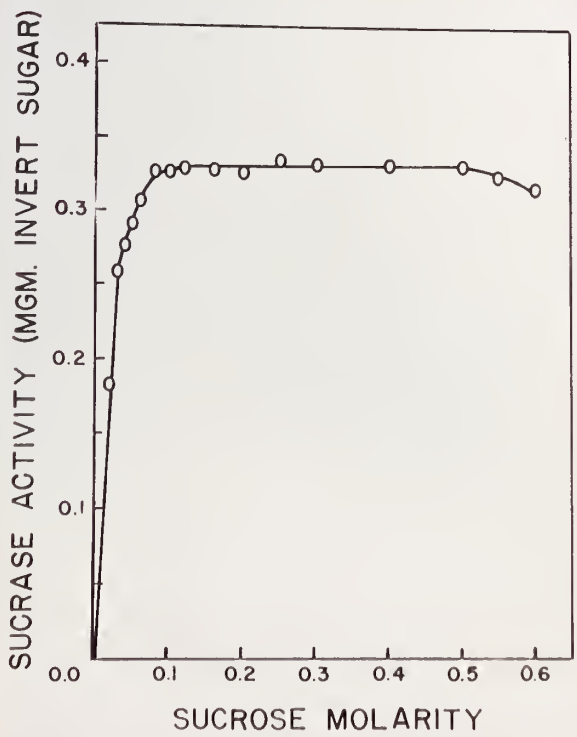

Fig. 4. The Effect of Substrate Concentration on the Rate of Intestinal Sucrase Action.

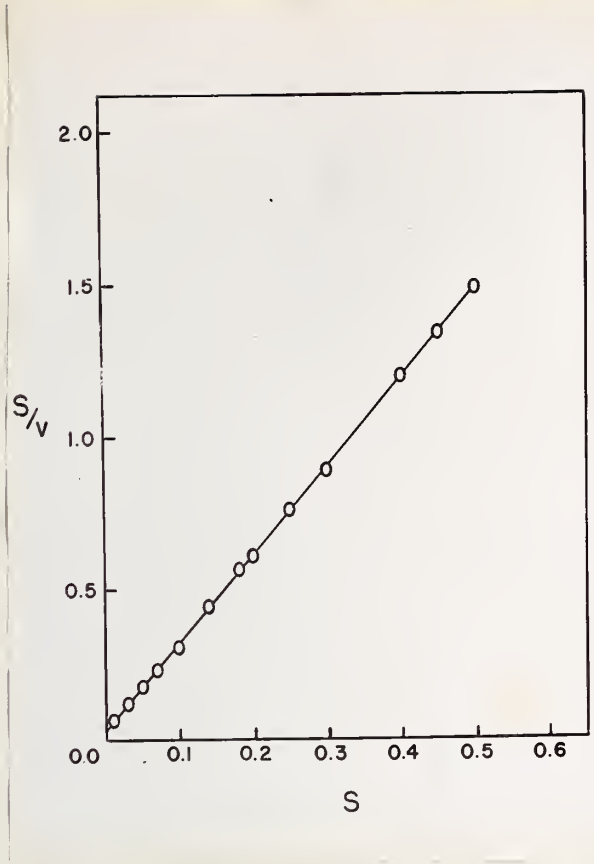

Fig. 5. The Determination of the Km Value for Intestinal Sucrase by a Lineweaver Plot of S/v vs. S.

S = substrate concentration = molarity of sucrose.

v = reaction velocity (mgm. invert sugar formed in 15 minutes).

S/v = substrate concentration ÷ reaction velocity.

The slope of the straight line = $\frac{1}{V_{max}}$ = 2.915

Ordinate intercept = $\frac{Km}{V_{max}}$ = 0.037

V_{max} = 0.343

Km = 0.0127 M.

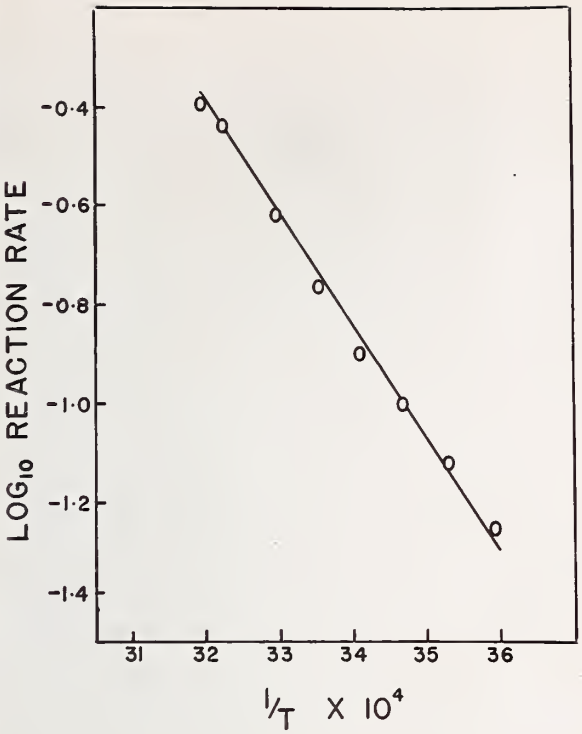

Fig. 6. The Effect of Temperature on the Rate of Intestinal Sucrase Action.

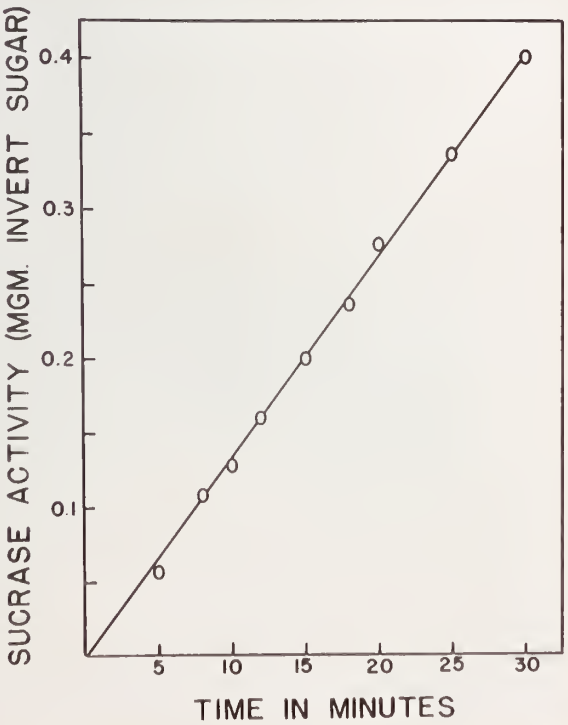

Fig. 7. The Effect of Time on the Rate of Intestinal Sucrase Action.

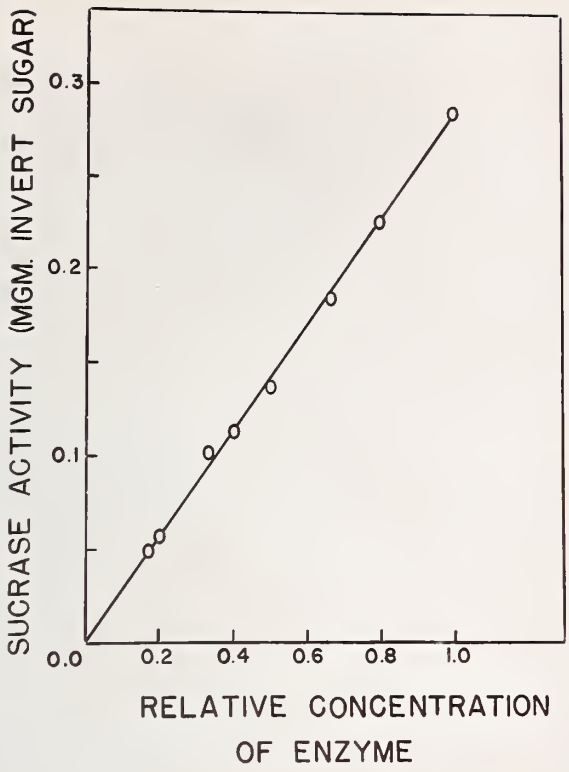

Fig. 8. The Effect of Enzyme Concentration on the Rate of Intestinal Sucrase Action.

The optimum pH was determined as 6.25 ± 0.15 (figure 3). This pH is the same as that determined when a homogenate of the first 10 cm. section of the intestine was used (ref. Sec. II, B, 3(i)).

In substrate concentrations of 0.12 M (4.1%) to 0.5 M (17.1%) sucrose, the enzyme action is maximal (figure 4). The Km value calculated algebraically from the data is 0.0128 M, whereas the value determined graphically is 0.0127 M. V_{max} determined algebraically = 0.349 mgm. invert sugar per fifteen minutes. The same value calculated from the slope ($\frac{1}{V_{max}}$ = 2.915) of the graph is 0.343 mgm. invert sugar produced in fifteen minutes. The experimental values for v compare favorably with the calculated values as is illustrated below:

S (Molar)	v, Experimental (Mgm. invert sugar produced in 15 min.)	v, Calculated (Mgm. invert sugar produced in 15 min.)
0.04	0.276	0.265
0.10	0.326	0.311
0.20	0.326	0.329

The activation energy, μ, calculated from the "best" straight line obtained by plotting $\log_{10}$ reaction rate against $\frac{1}{T} \times 10^4$ is equal to 10,100 calories between 5° and 37°C. (figure 6). The Q_{10} values, calculated from the experimental data of two trials, for 10° temperature ranges are presented below:

Temperature Range (°C.)	Q_{10}
5-15	1.74
10-20	1.67
15-25	1.70
20-30	1.81
25-35	1.79
30-40	1.57

A plot of reaction rate against time produced a straight line over a thirty-minute period for an undiluted 100 ml. intestinal homogenate (figure 7). When a 1:5 diluted homogenate was used a straight line relationship between time and reaction rate was found over a seventy-five minute period. When a

homogenate of the first 10 cm. section of the intestine was used previously, the time-reaction-rate relationship was linear over the twenty-minute period investigated. A 1:3 dilution of this homogenate produced a linear relationship for approximately 105 minutes.

A linear relationship between relative enzyme concentration and reaction rate proved to be constant over the range of homogenate dilutions studied (figure 8).

Rat intestinal sucrase is quite stable. No change was noted in the sucrase activity of intestinal homogenates kept in the refrigerator at 5°C. for thirty-seven days.

4. DISCUSSION

The results obtained from the study of the kinetics of rat intestinal sucrase action agreed favorably with those that one might have expected, both from the data in the literature and from preliminary experiments.

The optimum pH of 6.25 and the optimum sucrose concentration of 0.12 M to 0.5 M are of the same magnitude as the corresponding values used in the method developed for determining sucrase activity. Therefore, the method requires no revision.

In the study of the effect of pH on the rate of intestinal sucrase action, three buffer systems were used. The enzyme response noted at different pH's in one buffer system is characteristic of the enzyme only when that buffer is employed; that is, the activity noted in one buffer system would not necessarily be exhibited in another buffer of the same pH. However, the optimum pH of 6.25 is clearly defined within the range of the phosphate buffer only, and the optimum is within the pH 6-8 range usually cited for intestinal sucrase. An optimum pH of 6.25 is quite reasonable for rat intestinal sucrase, for the pH of the upper part of the small intestine of rats is 6, and at lower

levels the pH is around 6.8 (13). Rat intestinal sucrase activity falls off more slowly on the acid side of the pH curve than on the alkaline side (ref. figure 3), and it is similar to yeast invertase in this respect.

The finding that maximum sucrase activity occurs in sucrose concentrations of 0.12 M to 0.5 M agrees with the values found by Nelson and Vosburgh (68) for yeast invertase. However, the range of sucrose concentrations promoting maximum intestinal sucrase activity seems to be broader than the range (0.146 M to 0.292 M) for yeast invertase.

A μ value of 10,100 calories is approximately 1,000 calories less than the activation energy reported for yeast invertase by Sizer and Josephson (77). The lower value of μ indicates that rat intestinal sucrase is a better catalyst than yeast invertase, but one must remember that highly impure enzyme preparations were used in the investigations reported in this thesis.

The fact that sucrose inversion proceeds according to zero-order kinetics at high sucrose concentration was in accordance with expectations. The falling off of the zero-order reaction shortly after seventy-five minutes is probably due to heat inactivation of the enzyme. The decrease in activity is not due to a decrease in substrate concentration causing the enzyme to be no longer saturated, because the sucrose concentration is decreased by only 0.17 mg. per ml. or 0.017 per cent after ninety minutes. The decrease in activity may, however, be enhanced by the increased hydrolytic production of glucose, and α-glucose is an inhibitor of glucoinvertase activity (69).

5. SUMMARY

A study of the kinetics of rat intestinal sucrase action revealed the following data:

1. The optimum pH for the enzyme is 6.25 $\pm$ 0.15.

2. The sucrose concentrations for optimum enzyme activity are between 0.12 M and 0.50 M.

3. Km = 0.0128 M.

4. The activation energy, μ, is 10,100 calories between 5° and 37°C.

5. The reaction is zero order when the enzyme is saturated with substrate (0.161 M), and the zero-order reaction continues in effect for over thirty minutes when a 100 ml. homogenate is employed. The zero-order reaction lasts for at least 75 minutes when a 1:5 dilution of the homogenate is used.

6. Intestinal sucrase activity is directly proportional to enzyme concentration over the range of enzyme concentration studied.

7. Intestinal sucrase is stable for long periods of time.

V. THE EFFECT OF SEX AND AGE ON THE INTESTINAL SUCRASE ACTIVITY OF NORMAL RATS

1. INTRODUCTION

The literature contains no references to the effect of the sex and age of animals on the levels of rat intestinal sucrase. The enzyme is, however, known to occur in the intestinal tract of both the foetus (62) and the adult (70) of humans. The presence of the enzyme in the foetus shows that sucrase is not due to ingested food, although the enzyme may be influenced by the type of food ingested later.

In the case of serum alkaline phosphatase, Tuba, Baker, and Cantor (89) found that the activity of this enzyme was significantly higher in adult male rats than in adult females, and that the phosphatase activity varied directly with the food consumption. Tuba and Wiberg (96) found that serum amylase was significantly higher in adult male rats than in adult female rats. Similar effects might be observed for sucrase, since sucrase is a digestive enzyme and, therefore, it could be reasoned that the enzyme would vary in activity with the amount of food ingested.

2. EXPERIMENTAL

After it was found that the first half of the small intestine is the main locale of rat intestinal sucrase activity, the effect of the age and sex of the animals on the levels of the enzyme in the third 10 cm. section of the small intestine of normal rats was determined. Male and female rats seven months, three months, and twenty-three days old were selected for the experiment. Five seven-months-old lactating breeder females were also used. The other group of seven-months-old females consisted of animals which had had their litters weaned three to four weeks previous to the experiment.

Prior to being selected for the experiment, all the animals, with the exception of the weanling rats, had been maintained on ad libitum diets of Purina fox checkers and water. Each age group was composed of five rats, except for the group of seven-months-old males and the group of seven-months-old nonlactating females which were composed of six and four animals respectively.

The animals were weighed individually and then killed by decapitation. The small intestine was then excised intact and measured with a rule calibrated in centimeters. The third 10 cm. section of the intestine was next removed in the case of the three- and seven-months-old animals, but in order to keep the intestinal sections comparative, sections equivalent to one-eleventh of the length of the small intestine were taken from the weanling animals (23 days of age).

The intestinal sections were cleaned, weighed, and homogenized, and 1 ml. aliquots of the homogenates were assayed for sucrase activity (ref. Sec. II and III).

Statistical analyses were conducted to determine the degree of difference between the age groups within each sex, and between the sexes at the same age level. The results of these analyses are presented in Table XII (b).

3. RESULTS

The experimental data are presented in Table XII (a) and the results of the statistical analyses of these data are given in Table XII (b).

The data show incontestably that intestinal sucrase activity is the highest in the oldest animals. The differences between the enzyme activity of the oldest animals and that of the younger animals are highly significant.

Table XII (a)
The Effect of Sex and Age
on the Intestinal Sucrase Activity of Normal Rats

Sex	Age	Average Body Weight in grams	Average Length of Small Intestine in cm.	Length of 3rd proportionate Intestinal Section in cm.	Sucrase Activity in Units per gram of Wet Tissue Mean*	Range
**Male	7 months	367	126	10.0	52.2 ± 2.42	40.9 - 58.1
Male	3 months	197	116	10.0	32.3 ± 1.86	29.0 - 39.1
Male	23 days	41.6	74.7	6.8	34.4 ± 2.69	26.9 - 42.0
***Female	7 months (nonlactating breeder)	243	135	10.0	55.0 ± 2.67	50.5 - 61.2
Female	7 months (lactating breeder)	260	134	10.0	51.2 ± 1.37	46.0 - 54.1
Female	3 months (virgin)	134	106	10.0	31.9 ± 2.16	26.7 - 39.4
Female	23 days	44.2	73.1	6.7	39.8 ± 1.94	34.2 - 46.2

*Mean ± standard error of the mean.

**Values given for 7-months-old males are the averages for six animals.

***Values are the averages for four animals.

Table XII (b)
Statistical Analyses for Values
for Intestinal Sucrase Activity of
Male and Female Rats of Various Ages

Analysis	Groups	Value of "t"	Value of "P"	Significance*
Age of Males	7 months vs. 3 months	6.53	< 0.01	HS
	7 months vs. 23 days	4.92	< 0.01	HS
	3 months vs. 23 days	0.61	> 0.05	NS
Age of Females	7 months (nonlactating) vs. 7 months (lactating)	1.337	> 0.05	NS
	7 months (nonlactating) vs. 3 months	6.79	< 0.01	HS
	7 months (lactating) vs. 3 months	7.55	< 0.01	HS
	7 months (lactating) vs. 23 days	4.80	< 0.01	HS
	7 months (nonlactating) vs. 23 days	4.68	< 0.01	HS
	3 months vs. 23 days	2.72	0.02-0.05	S
Sexes	Males 7 months vs. non-lactating females 7 months	0.83	> 0.05	NS
	Males 7 months vs. lactating females 7 months	0.37	> 0.05	NS
	Males 3 months vs. Females 3 months	0.15	> 0.05	NS
	Males 23 days vs. females 23 days	1.65	> 0.05	NS

*HS = highly significant

S = significant

NS = nonsignificant

The sucrase levels of weanling rats and three-months-old rats are similar, although a higher range of values is noted in the case of the weanling female rats.

A difference between the enzyme levels of lactating and nonlactating female rats was surmised when the values for the lactating females, determined prior to those for the nonlactating rats, were compared with those of males of the same age. However, no significant difference is indicated.

4. DISCUSSION

The finding of relatively high levels of intestinal sucrase in weanling rats tends to indicate that the presence of the enzyme in the intestinal tract is not due to the ingestion of sucrose. The lactose of the mother's milk would be the chief carbohydrate which the weanling rats had received in their diet.

Higher levels of sucrase activity in the seven-months-old animals than in the younger ones, but little difference between the levels of the enzyme in the two age groups of younger animals, seem to suggest that the levels of sucrase originally present are high enough to accomplish the hydrolysis of the sucrose of the animals' Purina fox checker diet until the adult state is attained. No significant differences were found between the intestinal sucrase levels of male and female rats of the same age, indicating that neither the body weight nor the differences in food consumption accompanying the differences in weight directly influences the levels of the enzyme. Also, the results indicate that the sucrase activity in rats is the same in male and female animals of the same age. However, if the animals were compared at various weights, with the age then being variable, the sucrase activity of the females would probably then be found greater than that of the corresponding males, at least in the 250 to 400 gram weight range.

The eating habits of the animals constitute one factor which might influence the sucrase levels. However, this problem was at least partially controlled by killing the animals at the same time of day.

As mentioned previously, no difference was found between the intestinal sucrase activity of lactating females and that of nonlactating females. This finding further supports the conclusion that food consumption, as such, does not influence sucrase activity directly. If the lactating rats had had higher sucrase levels than those of the nonlactating females, one would have been led to believe that the food consumption exerted a direct effect on the enzyme activity, assuming that the former animals consume more food than the latter.

None of these results disproves the possibility of a dietary-hormonal mechanism controlling the intestinal sucrase levels. Indeed, it appears possible that such a mechanism causes the formation of new enzyme when the sucrose content of the diet reaches a certain level.

Finally, there is the question of the high levels of sucrase activity noticed in the case of the seven-months-old female rats, and also in the case of the male rats of the same age. The sucrase activity in the third 10 cm. section of the small intestine of male rats of similar weights to those used in this investigation was previously determined (ref. Sec. III) as 43.7 ± 2.22 units per gram of wet tissue, and the range of activity was 35.4 to 55.2 units. The extent of the range of activity determined in the present investigation is about the same as that found previously, but the range and the mean have been shifted toward the higher values. This shift is probably due to improved experimental techniques. The high levels of sucrase found in the case of the female rats are influenced by the weight of the intestinal sections which were composed of less muscle tissue than the sections from the corresponding males. An increase in intestinal section weight does not necessarily parallel increased

sucrase activity. Therefore, an error is introduced by relating enzyme activity to the wet weight of the intestine, and the values for sucrase activity are not strictly comparative.

5. SUMMARY

1. Intestinal sucrase activity is greater in seven-months-old rats than in younger rats.

2. Little difference was found between the sucrase levels in three-months-old rats and those of weanling rats.

3. When the sucrase activities of male and female rats of the same age were compared, no significant difference was found.

4. The intestinal sucrase activities of seven-months-old lactating and nonlactating female rats were similar.

5. It is suggested that the amount of food consumption has no direct relationship to intestinal sucrase activity, although food consumption and the nature of the diet probably are related to the enzyme levels.

VI. THE EFFECT OF DIET ON RAT INTESTINAL SUCRASE ACTIVITY

A. THE EFFECT OF FASTING ON RAT INTESTINAL SUCRASE ACTIVITY

1. INTRODUCTION

The effect of fasting on rat intestinal sucrase activity has not been extensively investigated. Tuba and Dickie (90) reported that a fast of five days resulted in a decrease in sucrase activity which was not, however, significantly lower than the enzyme activity in normal rats. The purpose of this investigation is to verify these findings, and to determine the effect on intestinal sucrase activity obtained by varying the length of the fasting period.

Many investigators in the Department of Biochemistry at the University of Alberta have demonstrated that enzyme activity in animal tissues is depleted when the animal is subjected to protein depletion of its tissues by fasting. Wiberg (106) has shown that the amylase activity of serum, pancreas, and intestinal mucosa is significantly decreased in fasted rats. It has also been demonstrated, here and at other laboratories, that the levels of intestinal and serum alkaline phosphatase in rats are significantly diminished upon fasting (18, 30, 57, 103).

Enzyme activity in the intestinal tract seems to be correlated with the nitrogen (protein) content of the tissue. For example, Robinson (75, 93) showed that the decreased intestinal alkaline phosphatase activity parallels the accompanying decrease in intestinal and liver nitrogen in rats. Rat intestinal phosphatase was depleted to approximately 67% of the normal value after two days' fast, and to 54% of the normal value after six to twelve days' fast. In connection with sucrase, Virtanen and Winkler (100) have noted the

decrease or disappearance of sucrase activity with decreased nitrogen in Escherichia coli. On the basis of these findings, a decrease in intestinal sucrase activity with decreased intestinal nitrogen would be anticipated. On the other hand, decreased intestinal nitrogen or protein need not necessarily mean a decrease in the activity of any one particular enzyme.

2. EXPERIMENTAL

Five groups of six adult male albino rats, weighing between 300 and 410 grams each, were fasted for various lengths of time (1, 2, 4, 8, and 12 days). During the fasting period the animals were housed in individual cages and received water ad libitum. At the termination of the fast the animals were weighed and then killed by decapitation. Then, the first 50 cm. of the small intestine, measured from the pylorus, was removed and cut into 10 cm. sections which were frozen until required.

A preliminary experiment was, first of all, carried out in order to find in which intestinal section the greatest and most consistent decrease in sucrase activity would occur when rats were fasted. Accordingly, the intestinal sections of one or two rats from each of the groups fasted for 1, 2, 4, and 8 days were cleaned and homogenized as described in Sec. III. Sucrase activity was determined by the dinitrosalicylic acid method. The intestinal homogenates were prepared so that 1 ml. aliquots produced invert sugar concentrations equivalent to per cent light transmissions of 10 to 70 at 510 mμ. The test tubes were centrifuged to produce clear solutions. After the results (table XIII) were studied, the third 10 cm. section of the small intestine was chosen for use in the following studies. Thus, homogenates of the third section from each of the remaining animals were prepared, and the sucrase activity was determined.

In order to measure the extent of protein depletion by fasting, the nitrogen content of the intestinal sections was determined by a macro-Kjeldahl method. Five or 10 ml. samples of homogenate were used.

The average intestinal sucrase activities for the animals fasted for the periods described were compared with the average intestinal sucrase activity of normal rats, and statistical analysis was used to determine the extent of difference from the normal value (table XIV). Also, the average sucrase activity for animals fasted for the various periods was compared to the average nitrogen content of the intestinal sections after the same period of fasting (table XV). The nitrogen content and sucrase activity were based on the initial body weight of the animals in order to allow for the weight loss undergone by the tissues.

3. RESULTS

Results of the fasting experiment are presented in tables XIII to XV.

The data clearly show that sucrase activity is decreased by fasting, and that the maximum decrease occurs after two to four days, after which time the activity apparently remains relatively constant even after the fasting period is extended to twelve days. Reference to table XV shows that the nitrogen and, therefore, protein content of the intestinal tissue decreases at a slower rate than does sucrase activity. Also, sucrase activity seems to reach a minimum after about two days' fast, and the activity remains at that level even though the nitrogen content of the intestinal tissue continues to decline as the fasting period is extended. Thus, sucrase activity does not directly parallel the nitrogen or protein content of the tissue, although there is a definite relationship between sucrase activity and decreased nitrogen.

Table XIII
The Intestinal Sucrase Activity (Units per Gram of Wet Tissue)
in the First Five 10 cm. Sections
of the Small Intestine of Fasted Rats
(Figures in Parentheses Represent the Per Cent of Normal Activity.)

Fasting Period in Days	Section of Intestine				
	1	2	3	4	5
*0	31.1 (100)	46.9 (100)	43.7 (100)	43.2 (100)	41.1 (100)
**1	24.3 (78)	37.6 (80)	34.6 (79)	29.4 (68)	27.2 (65)
2	18.3 (59)	30.0 (64)	21.7 (50)	22.7 (53)	25.7 (62)
**4	17.4 (56)	19.8 (42)	15.9 (36)	--	16.2 (39)
8	20.2 (65)	22.0 (47)	**15.3 (35)	**15.2 (35)	20.5 (48)

*Values for unfasted animals are the means for ten animals.

**Values for animals fasted for one and four days, and for the third and fourth intestinal sections of animals fasted for eight days are the values for single animals. All other values are the means for two animals.

Table XIV

The Intestinal Sucrase Activity of Rats Fasted for Varying Periods of Time

Length of Fast in Days	Average Body Weight in Grams		Sucrase Activity in Units per Gram of Wet Tissue*	Per Cent of Normal Activity	"P"	Sucrase Activity in Units per 100 Grams of Initial Body Weight*	Per Cent of Normal Activity	"P"
	Initial	Final						
**0	375	375	43.7 ± 2.22	100.0	-	7.71 ± 0.60	100.0	-
1	355	332	32.9 ± 2.55	75.3	<0.01	5.44 ± 0.58	70.5	<0.01
2	348	311	25.6 ± 1.67	58.0	<0.01	3.67 ± 0.14	47.6	<0.01
4	328	263	19.6 ± 1.58	44.8	<0.01	2.51 ± 0.25	32.5	<0.01
***8	354	261	19.7 ± 2.29	45.1	<0.01	2.25 ± 0.16	29.2	<0.01
12	367	238	23.5 ± 0.78	53.6	<0.01	2.11 ± 0.22	27.3	<0.01

*Mean ± standard error of the mean.

**Values are the means for ten animals.

***Values are the means for five animals. All other values are the means for six animals.

Table XV

The Relationship Between Intestinal Sucrase Activity and Intestinal Nitrogen of Rats Fasted for Varying Periods of Time

Length of Fast in Days	Average Initial Body Weight in Grams	Sucrase Activity in Units per 100 Grams of Initial Body Weight*	Per Cent of Normal Activity	Mgm. N per 100 Grams of Initial Body Weight*	Per Cent of Normal	Units of Sucrase Activity per Mgm. N
**0	375	7.71 ± 0.60	100.0	4.66 ± 0.20	100.0	1.65
1	355	5.44 ± 0.58	70.5	4.15 ± 0.30	89.0	1.31
2	348	3.67 ± 0.14	47.6	3.08 ± 0.16	66.2	1.19
4	328	2.51 ± 0.25	32.5	2.99 ± 0.25	64.2	0.84
***8	354	2.25 ± 0.16	29.2	2.53 ± 0.18	54.3	0.89
12	367	2.11 ± 0.22	27.3	2.06 ± 0.23	44.2	1.02

*Mean ± the standard error of the mean.

**Values are the means for ten animals.

***Values are the means for five animals. All other values are the means for six animals.

4. DISCUSSION

The significant decrease in rat intestinal sucrase activity after a fasting period of as little as one day is in disagreement with the earlier findings of Tuba and Dickie (90). The discrepancy may be due to the differences (ref. Sec. III) in the methods of the sucrase assay, or to the use of the first 10 cm. section of the small intestine in the previous study.

The data indicate that intestinal sucrase acts like intestinal alkaline phosphatase in the fasted rat. The enzyme activity falls to a minimum in two to four days and remains at that level even after fasting is prolonged. This result was in accord with expectations.

Sucrase activity seems to be definitely correlated with the nitrogen or protein content of the intestine, although there is no direct parallel between the levels of enzyme activity and the nitrogen content of the tissue. The absence of a direct parallel suggests that the enzyme protein is depleted like the general protein of the tissue until a minimum level of enzyme is reached. When this minimum enzyme level is attained, protein depletion of nonenzyme protein apparently continues since the nitrogen content of the intestinal tissue continues to be decreased with increased fasting. However, many undetermined factors, as well as nitrogen or protein depletion, may affect enzyme activity, and one must remember that enzyme activity and enzyme content are not necessarily equal entities.

5. SUMMARY

1. Intestinal sucrase activity of rats fasted for 1, 2, 4, 8, and 12 days is significantly decreased from the normal value.

2. The maximum decrease in sucrase activity occurs after 2 to 4 days' fast, and the enzyme activity remains at the minimum level during extended periods of fasting.

3. There is a definite relationship between intestinal sucrase activity and intestinal nitrogen although there seems to be no direct correlation between the entities.

B. THE EFFECT OF THE SUCROSE CONTENT OF THE DIET ON RAT INTESTINAL SUCRASE ACTIVITY

1. INTRODUCTION

The effect that the sucrose content of the diet has on rat intestinal sucrase activity is unknown. If one thinks in terms of enzymic adaptation, he would anticipate a decrease from the normal enzyme activity when a diet lacking sucrose is fed, and anticipate an increase in activity when a diet containing more sucrose than the normal diet is fed.

Many investigators have observed changes in the enzyme content of animal tissues following the ingestion of various diets. Grossman, Greengold, and Ivy (38) found that diets high in carbohydrate or protein produced marked changes in some of the pancreatic enzymes of the rat after 21 days. Wiberg has shown that rat serum amylase is significantly altered by diets high in protein or fat, and by diets lacking available carbohydrate (106); while other workers in the Department of Biochemistry have shown that serum lipase and alkaline phosphatase respond to changes in the diet (88, 92, 94, 95). With regard to β-h-fructosidase (yeast sucrase), the amount of the enzyme in yeast can be increased by incubating the yeast with small amounts of sucrose (85). More relevant to the present study is the work of Ugo Lombroso (56), who observed no increase in the sucrose hydrolyzing power of the intestine when sucrose was perfused into the intestinal lumen of dogs in large amounts and concentrations. The same phenomenon may perhaps occur in rats. Also, Euler and Svanberg (26) conclude that enough sucrase is present in the human intestinal wall to digest the amount of sucrose normally ingested.

Although the changes in enzyme activity apparently produced by changes

in an animal's diet do not constitute true enzymic adaptation in that no new (i.e. different species of enzyme) enzyme is added to the normal enzyme pattern, they are, nevertheless, quantitative changes in the amount of an enzyme already present. These changes in the amount or activity of enzymes present in animal tissues may be considered a form of enzymic adaptation.

The present experiment was designed to determine the response of rat intestinal sucrase to diets containing varying amounts of sucrose as the available carbohydrate.

2. EXPERIMENTAL

Three groups of twelve adult male albino rats, weighing between 255 and 375 grams each, were selected for the experiment. Preliminary to being placed on experimental diets, the animals received ground Purina fox checkers and water ad libitum for a period of one week. The three groups of rats were subdivided into three subgroups of four animals each. One subgroup in each group was fed one of the three experimental diets for a period of one, two or four weeks. In addition, another subgroup of four rats was killed at the end of the one-week stabilization period in order to obtain initial control values. The average weight of the animals in the subgroups was 315 grams. All the animals were housed in individual cages and received water and food ad libitum throughout the course of the experiment.

The composition of the three diets was as follows:

(1) Control Diet of Purina Fox Checkers (Ralston Purina Company of Canada, Ltd.)

Guaranteed Analysis:

Crude fat - not less than 3%
Crude protein - not less than 20%
Crude fibre - not more than 6%

(2) High Sucrose Diet

Fat (Crisco)	8%
Sucrose	70%
*Casein	18%
**McCollum-Davis Salt Mix	4%
***Vitamins	

(3) Low Sucrose Diet

Fat (Crisco)	8%
*Casein	88%
**McCollum-Davis Salt Mix	4%
***Vitamins	

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*Casein (General Biochemicals, Inc.)
89% casein, 0.5% fat, 0.0% lactose

**McCollum-Davis Salt Mix No. 185 (J. Biol. Chem., 33: 55, 1918)

Calcium lactate	35.15 gm.
Ca $(H_2PO_4)_2 \cdot H_2O$	14.60
K HPO_4	25.78
$NaH_2PO_4 \cdot H_2O$	9.38
NaCl	4.67
$MgSO_4$ (anhydrous)	7.19
Ferric citrate	3.19

***Vitamins (quantities per kilogram of ration)

Thiamine hydrochloride	5 mgm.
Pyridoxine hydrochloride	5 mgm.
Niacin	5 mgm.
Calcium pantothenate	20 mgm.
Riboflavin	10 mgm.
Choline chloride	1 gm.
Vitamin A acetate	1.3 mgm.
Calciferol	0.15 mgm.
α-tocopherol	120 mgm.
Ascorbic acid (antioxidant for thiamine)	500 mgm.

(Kandutsch, A.A. and Bauman, C.A. J. Nutrition, 49: 209 (1953).)

At the termination of the test the animals were weighed and then killed by decapitation. The third 10 cm. section of the small intestine was removed from each animal and homogenized as described in Sec. III. Intestinal sucrase activity was determined, and the values for the test groups were compared with those for the control groups. Statistical analysis was carried out in order to determine the significance of the differences existing between the sucrase activities of the various groups.

3. RESULTS

The results of the experiment are presented in table XVI.

Table XVI
The Intestinal Sucrase Activity of Rats Fed Diets Containing Varying Amounts of Sucrose

Time on Diet	Average Body Weight in Grams Initial	Final	Average Daily Food Consumption in Grams	Sucrase Activity in Units per Gram of Wet Tissue
(1) Purina Fox Checkers (Control)				
Initial 1-week stabilization period	315 ± 22*	320 ± 22*	18.2 ± 1.35*	43.8 ± 5.17*
1 week	329 ± 17	388 ± 13	19.6 ± 0.70	42.8 ± 2.61
2 weeks	316 ± 25	335 ± 21	20.7 ± 0.91	47.6 ± 4.23
4 weeks	322 ± 21	348 ± 18	21.4 ± 1.27	48.4 ± 2.56
(2) High Sucrose (70%)				
1 week	308 ± 24	343 ± 22	15.8 ± 0.70	40.9 ± 2.23
2 weeks	312 ± 20	325 ± 19	16.4 ± 0.60	61.4 ± 4.71
4 weeks	310 ± 23	326 ± 22	16.0 ± 0.70	53.2 ± 2.69
(3) Low Sucrose (0%)				
1 week	315 ± 15	313 ± 13	13.1 ± 0.40	23.0 ± 0.99
2 weeks	310 ± 22	310 ± 21	13.5 ± 1.66	27.5 ± 2.48
4 weeks	313 ± 19	315 ± 20	13.8 ± 1.09	25.4 ± 2.29

*Standard error of the mean.

There is a highly significant ($P < 0.01$) difference between the sucrase activity of rats fed the low sucrose diet for one, two, or four weeks, and the enzyme activity of rats fed the control diet of fox checkers. Similarly, there is the same significance between the sucrase activity of animals fed the low sucrose diet and the sucrase activity of animals fed the high sucrose diet. However, there is no significant difference ($P > 0.05$) between the activity in the group of rats receiving the high sucrose diet and the activity in animals fed the control diet. Also, there is no significant difference between the values for the four subgroups of control animals, or between the values for the subgroups in the groups receiving the high or low sucrose diets.

The results, thus, show decisively that sucrase activity remains at the normal level under the influence of a high sucrose diet, and is significantly decreased under the influence of a diet which lacks sucrose but has a high protein content. The significant decrease in sucrase activity under the influence of the latter diet occurred after the animals had been fed the diet for only one week, and the activity remained at the low level for the duration of the diet, i.e., four weeks. Reference to Sec. VI, A, will show that the sucrase activity obtained with the sucrose-deficient diet is the same as that obtained by two days of fasting.

The fact that there was little difference between the food consumptions of the animals on the test diets prevents the possibility of there being a difference in sucrase activity due to a difference in food consumption.

4. DISCUSSION

The normal values for intestinal sucrase activity obtained with the high sucrose diet were not unexpected. An explanation of the phenomenon may be that the intestinal mucosa of the rat contains enough sucrase to take care

of concentrations of sucrose much larger than those normally consumed by the animals. This result agrees with that observed by Ugo Lombroso (56) in dogs.

The decrease in the intestinal sucrase activity of rats fed a sucrose-deficient diet indicates that production of the enzyme may be stimulated from a basic level by ingested sucrose. With the high protein diet employed there is no possibility of protein or amino acids not being available for protein and enzyme synthesis. The decrease in sucrase activity appears to be the result of enzymic adaptation in response to lowered substrate concentration.

5. SUMMARY

1. A high sucrose diet (70% sucrose) had the same effect on intestinal sucrase activity in rats as a control diet of Purina fox checkers.

2. A diet which lacked sucrose, but which contained a large amount of protein, decreased rat intestinal sucrase activity below the activity obtained with a diet of Purina fox checkers. The phenomenon may perhaps be due to an enzymic adaptation.

C. THE RESPONSE OF INTESTINAL SUCRASE OF FASTED RATS TO VARIOUS DIETARY SUGARS

1. INTRODUCTION

No studies have been conducted to determine the response of rat intestinal sucrase to various sugar diets ingested by fasted animals. The results obtained in the preceding experiment (Sec. VI, B) suggest that intestinal sucrase levels may be increased from a basic level by sucrose ingestion, and the question of the effects of other sugars, such as glucose, fructose, invert sugar, maltose, and lactose, also arises.

On the basis of enzyme specificity and enzymic adaptation, one would expect sucrose and melizitose to stimulate the production of intestinal sucrase, whereas α-glucose might be expected to exert an inhibitory action (69). One would not expect fructose, xylose, maltose, or lactose to influence sucrase production.

If it could be shown that maltose, in contrast to sucrose, has no influence on sucrase activity, Weidenhagen's view that sucrase and maltase are identical (102) would be discredited. On the other hand, if sucrose and maltose exert the same influence on sucrase production the theory that the two enzymes are one and the same would not necessarily be proven.

The stimulation of the production of the intestinal enzyme, alkaline phosphatase, in fasted rats by the feeding of fats and fatty acids (23, 93), certain carbohydrates (90), and some proteins (91) is well recognized. The present investigation was initiated in order to ascertain whether or not intestinal sucrase also adapts to the presence of substrate in the diet.

2. EXPERIMENTAL

Adult male albino rats, weighing between 190 and 310 grams each, were used for this investigation. The animals, housed in individual cages, received water ad libitum throughout the fasting and feeding periods. Prior to being selected for the experiment the animals had been maintained on an ad libitum diet of Purina fox checkers and water.

Many preliminary experiments were conducted in order to find a diet which could be used as a control, and to find a method for determining the effects of the dietary sugar to be tested. Since the preceding experiment (Sec. VI, B) had shown that a diet lacking sucrose, but containing 88% casein, caused a decrease in sucrase levels, the same diet was selected for use as the control regimen in the present investigation.

At first a fasting period of two days was chosen, and it was found that a high sucrose diet produced a substantial increase in sucrase activity above the two-day fasting level when the diet was fed ad libitum for 24 hours to the fasted animals. Then 5 grams of the same diet were offered to animals at the end of the two-day fast, and the sucrase activity was determined 4, 6, 8 and 10 hours after the diet was offered. It was found that the optimum time for killing the animals was six hours. However, the sucrase activity in animals fasted for two days was found to have a relatively large range and, consequently, the fasting period was extended to three days in order to try to obtain more consistent basal values. The time for the sucrase activity to reach a maximum after food ingestion, following fasting, was again determined and found to be 10 to 12 hours. The more suitable 10-hour period was chosen for use in the investigation.

However, when the sucrose deficient-high casein diet was fed, the sucrase level was elevated to the same level obtained with the high sucrose diet. Since relatively impure casein (ref. Sec. VI, B) had been used to prepare the sucrose-deficient diet, a similar diet containing vitamin-free casein was prepared and fed to animals. Results similar to those obtained with the first sucrose-free diet were found. Similarly, a diet of 100% vitamin-free casein, and a diet containing equal amounts of fructose and glucose (35% of each) produced the same results. The procedure for feeding the diets was consequently changed.

The sucrose free-high casein diet had significantly lowered the intestinal sucrase activity in rats within one week. Therefore, groups of rats were now fed the diet for one, two and three days in order to determine how soon the decrease in sucrase activity occurred. The decrease occurred in one day, and since the decrease occurred so soon it was decided that feeding the diet ad libitum for 24 hours after a fast of three days should

produce no increase in the enzyme level. This was found to be true, and, furthermore, when a high fat (45%) diet was similarly fed to rats fasted for three days there was no increase in sucrase activity. Therefore, a fasting period of three days, followed by ad libitum feeding of the test diets, and the use of the sucrose free-high casein diet as a control were adopted to test the effect of sugars on the production of intestinal sucrase.

Diets composed of 70% of the sugar to be tested, 18% casein, 4% McCollum-Davis salt mix, 8% Crisco, and vitamins as indicated in Sec. VI, B, were prepared to test the effect of various sugars on sucrase production. The following sugars were tested:

1. Sucrose - cane sugar.
2. Maltose - D(+) Maltose. C.P. Pfanstiehl Chemical Co.
3. Lactose - Lactose Merck Reagent. Merck and Co., Inc.
4. Glucose - D(+) Glucose. C.P., anhydrous. Pfanstiehl Chemical Co.
5. Fructose - D(-) levulose. Practical. Pfanstiehl Chemical Co.
6. Fructose + glucose (1:1 mixture).
7. Xylose - D(+) Xylose. Technical. Pfanstiehl Chemical Co.

As mentioned previously, the control diet contained 88% casein plus fat, minerals, and vitamins in the amounts indicated above. The high fat diet tested contained 45% Crisco, 51% casein, and minerals and vitamins.

Groups of five adult male albino rats, weighing between 220 and 310 grams each, were used in this part of the experiment, and the average weights of the groups were kept as close together as possible. The animals were fasted for three days, during which water was available ad libitum, and then they received the test diet plus water ad libitum for 24 hours. Because the diets varied in their palatability the same amount of food was not eaten in each case, but the quantities ingested were nearly the same in most cases.

At the termination of the feeding period, the animals were weighed and then killed by decapitation. The third 10 cm. section of the small intestine was excised in each case and then cleaned, weighed, and homogenized. A 1 ml. aliquot of the resultant 100 ml. homogenate was used for the determination of sucrase activity according to the method described in Sec. III.

Statistical analysis was applied to the results to determine the degree of difference between the test values and the value for the control.

The high levels of sucrase activity obtained ten hours after the feeding of the sucrose free-high casein diet to fasted rats was thought to be due to mechanical stimulation, since it is well known that mechanical stimuli increase the volume (71, 78) and also the total enzyme output of the juice of the small intestine (71). This increase of sucrase in intestinal juice may reflect prior increased enzyme formation. In order to test this hypothesis 5 grams of the high fat (45% Crisco) diet and 5 grams of the high glucose diet were similarly fed, and, in addition, 6 ml. each of distilled water, olive oil, and 58.3% sucrose (6 ml. $\cong$ 3.5 gm. sucrose) were force-fed to fasted animals from a 10 cc. hypodermic syringe fitted with a catheter (20). The results obtained were conflicting and inconclusive. Force-fed water produced a significant increase in the levels of the enzyme, but force-fed olive oil and sucrose produced no increase in activity above the fasting level of the enzyme. The high fat diet failed to have any stimulative effect, whereas the high glucose diet produced a substantial increase in sucrase activity.

Force-feeding of olive oil and sucrose resulted in diarrhea so that any stimulative effect of these substances on sucrase production would be counteracted, to some extent, by the loss of the foodstuffs and enzymes from the gastrointestinal tract. Also, in these instances the intestinal sections

seemed to be more fragile than they are normally. Increased fragility might mean that more than the usual amount of sucrase would be lost during the washing process. The hypertonicity of the sucrose solution would undoubtedly cause some dehydration of tissue cells and, therefore, influence enzyme levels. Water alone, on the other hand, was not lost and presumably is rapidly, but gradually, absorbed from both the small and large intestines.

The high fat diet seemed to have an inhibitory effect on intestinal sucrase production. This conclusion is suggested by the fact that the diet produced no increase in sucrase activity when fed in five gram quantities for ten hours, or ad libitum for 24 hours, even though the high casein diet caused a substantial rise in sucrase levels after ten hours. A factor of diet palatability is also involved, since the high fat diet was not readily consumed by the animals in this instance.

The fact that there was no increase in the intestinal sucrase levels of rats fed the high casein diet ad libitum for 24 hours after fasting is probably due to a decreased response to prolonged mechanical stimulation. A progressive fall in the enzyme concentration in the intestinal juice with continued mechanical stimulation has been observed both in dogs and in humans (71). Mechanical stimulation has been considered capable only of liberating enzymes from stores preformed in the mucosal cells, new formation depending on specific chemical-hormonal effects associated with food (71). However, the question of whether sucrase is actually secreted has not been solved, the generally accepted idea being that the enzyme in the succus entericus arises from shed epithelial cells of the intestinal mucosa (31, 79).

The phenomena observed might be explained by food, including water, in the intestinal lumen causing an initial mechanical stimulation of sucrase production and of sucrase release into the lumen, followed by a gradual

decrease in production, and by a more gradual decrease in the amount of the enzyme released, under prolonged stimulus lacking the proper dietary factors to stimulate new formation of the enzyme. In this way the initially produced enzyme would be gradually depleted to the fasting level by diets such as the high casein-sucrose free diet.

3. RESULTS

The results of the preliminary experiments conducted in studying the effects of sugars on intestinal sucrase are reported in Table XVII. Data for the comparative effects of the sugars, together with the results of statistical analyses, are presented in Table XVIII. Values of sucrase levels obtained with the high fat diet, and after a fasting period of three days, are also presented as additional control values in the latter table.

Sucrose was the only sugar which produced a highly significant increase in sucrase levels. Dietary fructose and maltose, and the diet containing equal amounts of glucose and fructose caused significant increases in the enzyme levels, but dietary glucose, xylose, and lactose produced no statistically significant enhancement of the enzyme activity.

Table XVII
The Effects of Various Experimental Factors on the Levels of Rat Intestinal Sucrase

Number of Animals	Preliminary Fasting Period in Days	Treatment	Average Food Consumption in Grams per Animal	Sucrase Activity in Units per Gram of Wet Tissue
2	2	Two days fast only	-	31.1
2	2	High sucrose diet ad lib. for 24 hours	16.0	52.0
2	2	5 gm. high sucrose diet for 4 hours	3.5	35.7
2	2	5 gm. high sucrose diet for 6 hours	4.5	46.5
2	2	5 gm. high sucrose diet for 8 hours	5.0	42.6
2	2	5 gm. high sucrose diet for 24 hours	5.0	26.9
7	3	Three days fast only	-	26.9
4	3	5 gm. high sucrose diet for 4 hours	3.0	35.9
4	3	5 gm. high sucrose diet for 6 hours	4.4	36.8
3	3	5 gm. high sucrose diet for 8 hours	4.1	39.3
4	3	5 gm. high sucrose diet for 10 hours	5.0	46.8
4	3	5 gm. high sucrose diet for 12 hours	5.0	49.4
4	0	High protein diet ad lib. for 1 day	14	31.4
4	0	High protein diet ad lib. for 2 days	22	35.2

Table XVII (continued)

Number of Animals	Preliminary Fasting Period in Days	Treatment	Average Food Consumption in Grams per Animal	Sucrase Activity in Units per Gram of Wet Tissue
3	0	High protein diet ad lib. for 3 days	35	29.4
4	3	5 gm. high casein (impure)diet for 10 hours	5.0	39.1
5	3	5 gm. high casein (vitamin-free) diet for 10 hours	5.0	39.0
5	3	5 gm. vitamin-free casein for 10 hours	3.2	47.7
4	3	5 gm. high fat diet for 10 hours	3.2	30.6
4	3	5 gm. 35% glucose-35% fructose diet for 10 hours	5.0	46.1
4	3	5 gm. high glucose diet for 10 hours	5.0	45.0
4	3	6 ml. H_2O force-fed. Animals killed in 10 hours		37.2
6	3	6 ml. 58.3% sucrose force-fed. Animals killed in 10 hours.		29.2
2	3	6 ml. olive oil force-fed. Animals killed in 10 hours		19.8

Table XVIII
The Effect of Various Dietary Sugars
on Levels of Rat Intestinal Sucrase when Fed for 24 Hours
to Rats Previously Fasted for Three Days

Dietary Regimen	No. of Animals	Average Initial Body Weight in Grams	Average Food Consumption in Grams per Animal	Sucrase Activity in Units per Gram of Wet Tissue*	**Value of "t"	Value of "P"
3-day fast	7	229	-	26.9 ± 1.34	0.657	> 0.05
Sucrose free-high casein diet	5	254	13.6	28.5 ± 2.05	-	-
Sucrose free-high fat diet	5	234	18.2	26.0 ± 1.48	0.473	> 0.05
70% sucrose	5	280	15.4	50.2 ± 3.97	4.86	< 0.01
70% maltose	5	246	17.0	39.4 ± 3.01	3.00	0.01-0.02
70% lactose	5	249	7.8	37.1 ± 3.75	2.00	> 0.05
70% glucose	5	236	13.6	32.4 ± 3.10	1.58	> 0.05
70% fructose	5	256	12.0	45.0 ± 4.73	3.21	0.01-0.02
35% glucose-35% fructose	5	252	13.4	39.2 ± 2.68	3.18	0.01-0.02
70% xylose	5	264	10.0	27.8 ± 1.70	0.263	> 0.05

*Mean ± standard error of the mean.

**Obtained by comparison with the sucrose free-high casein diet used as the control.

4. DISCUSSION

The highly significant rise in sucrase levels obtained with sucrose was in agreement with predicted results. As mentioned previously (Sec. VI, B) it has been observed that sucrose stimulates the production of sucrase in yeast.

The effect produced by glucose is also reasonable when one considers that glucoinvertase activity is inhibited by α-glucose, but not by β-glucose or β-fructose (69, 86). Nonstimulation of enzyme production and inhibition of enzyme activity are, of course, very different phenomena.

Fructose affords a very interesting result, since, unlike glucose, it produced a significant increase in the levels of sucrase. The reason why fructose should produce results different from those obtained with glucose is unknown. One reason that might be advanced, of course, is its uninhibitive action toward glucoinvertase activity. This result is similar to that observed with glucose in the case of pancreatic amylase. The hydrolytic product of starch (i.e. glucose) is said to increase the amylase content of the pancreas, but the products of casein have no such action on trypsin production (11).

Considering the results obtained with glucose and fructose individually, one would anticipate the intermediate enzyme levels obtained with the diet containing equal amounts of glucose and fructose. It appears that sucrase was synthesized, and the synthesis of the enzyme seems correlated to the amount of fructose in the diet.

The stimulation of sucrase production by maltose may be due to the similarity between maltase, an α-glucosidase, and intestinal sucrase (glucoinvertase), also an α-glucosidase. Both enzymes attack the α-glucosidic linkage in the substrate molecule, so it is not surprising to find that the substrate for one enzyme influences the production of the other. The influence of maltose on intestinal sucrase levels does not prove that intestinal sucrase and maltase are the same enzyme. More investigation needs to be conducted in

order to settle the question of the identify of the two enzymes. Lactase, on the other hand, does not attack α-glucosidic linkages, but β-galactosidic linkages (84), and, therefore, lactose has no significant influence on sucrase production.

Xylose caused no increase in sucrase production in strict accordance with the predicted results. Xylose is not acted upon by any intestinal enzymes, and it is usually not absorbed from the intestinal lumen.

The differences in the amounts of food ingested by the animals must be seriously considered in the case of the xylose and lactose diets which were not readily eaten by the animals. However, no correlation was seen between food consumption and enzyme activity for any one diet, and, furthermore, high food consumption does not parallel high sucrase activity for the different diets tested.

In conclusion, it would seem that sucrose and, to a lesser extent, maltose and fructose, stimulate the production of intestinal sucrase from a basic fasting level.

5. SUMMARY

1. It was found that a sucrose free-high casein diet caused an increase in intestinal sucrase levels in rats fasted for three days when fed for ten hours after the fasting period. The same diet, however, permitted a return to fasting levels of the enzyme when fed for 24 hours to animals previously fasted for three days.

2. Diets containing high amounts (70%) of various sugars were fed to rats for 24 hours after a three-day fasting period. Sucrose produced a highly significant increase in the levels of sucrase in the intestine, whereas dietary maltose and fructose, and a diet containing equal amounts of fructose and glucose, produced significant increases. Dietary glucose, lactose, and xylose failed to cause significant increases in the enzyme levels.

3. An initial mechanical stimulation of enzyme production was suggested to explain the increased sucrase levels observed when the sucrose free-high casein diet was fed for ten hours after a three-day fasting period.

VII. THE DISTRIBUTION OF SUCRASE IN RAT TISSUES

1. INTRODUCTION

Sucrase is not widely distributed in vertebrate tissues (69), and the enzyme seems to be confined to the mucosa of the small intestine of most vertebrates. However, no sucrase has been demonstrated in the small intestine of cattle (29) or newborn pigs (3). The mucosa of the intestine of pigeons (9) and the gizzard (69) and intestine (73) of chickens contain sucrase.

Neither saliva (69, 110) nor gastric juice (69) of vertebrates exhibits sucrase activity. The existence of the enzyme in cecal and colonic juice has been claimed by Vella (98). Supporting evidence has been given by Strashesko (80), who found sucrase in the cecal secretion, and by Maestrini (58), who found sucrase was sometimes present throughout the colon in sheep. Florey, Wright, and Jennings (31) conclude that it is doubtful whether sucrase, if it exists in the cecal and colonic juices, could perform any useful function. It is quite possible that the sucrase activity described in the cases above is derived from the small intestine by the seepage of succus entericus containing sucrase into the large intestine, and by the adhesion of the enzyme and/or cast-off mucosal cells to the feces.

Sucrase has been demonstrated in certain cysts of ovaries (53) as well as in the small intestine of the human adult (70), and also in the intestine of the fetus (62, 43) and stillborn (47, 87).

The association of sucrase with the circulatory system has not been clearly substantiated. In 1915 Domenico Lo Monaco (63) reported that the presence of the enzyme in the blood of normal and depancreatized dogs could not be demonstrated, but hyperglycemia always resulted after the injection of sucrose. In the same year Ugo Lombroso (55) was also unable to demonstrate

sucrase activity in defibrinated blood of the ox or dog. However, after intravenous injections of sucrose were made, Lombroso demonstrated that serum acquired slight sucrose hydrolyzing power. Rabbit lymph is said to possess sucrase (44). Morris and Boggs (65) were unable to demonstrate the presence of sucrase in leucocytes obtained from subjects suffering from leukemia. This result was contested by Boissevain (15), who contended that leucocytes contain sucrase and advanced the theory that the presence of the enzyme in serum after the injection of solutions of various sugars is due to injury to the leucocytes and the subsequent release of sucrase into the serum. Earlier workers had shown that sucrase occurs occasionally in the blood of young animals after sucrose injection (1, 104). Röhmann (76) suggested that this phenomenon was due to the gradual mobilization of the sucrase of the intestine rather than to new formation. The problem has not been resolved.

As noted previously, little information is available regarding sucrase activity in the rat. Thus, the distribution of the enzyme in rat tissues other than the wall of the small intestine (90) is unknown, and the present experiment was carried out in order to find if the distribution of the enzyme in the rat is similar to the distribution in the tissues of other vertebrates. On the basis of the known tissue distribution of sucrase, the possible presence of the enzyme was postulated only in the small intestine, caecum, large intestine, and leucocytes of the normal rat.

2. EXPERIMENTAL

The tissues to be examined for sucrase activity were obtained from normal male albino rats weighing approximately 300 grams each.

The animals were killed by decapitation, and the required tissues were excised, placed in ice-cooled beakers, and frozen in the freezing compartment

of the refrigerator until the time of assay. At that time the tissues were cleaned of connective tissue, washed with cold distilled water, patted dry with filter paper, and then weighed. The weighed tissue was then homogenized in cold distilled water in an ice-cooled Potter-Elvehjem glass homogenizer, and the resulting homogenate was made up to volume with cold distilled water in a volumetric flask.

Blood samples were collected from decapitated rats and were allowed to clot at room temperature in slanting test tubes containing no anticoagulant. The clotted blood was then chilled in the refrigerator overnight with the test tubes in an upright position. In the morning the serum was poured off from the side of the tube opposite the slanting clot. Then the serum was diluted 1 in 5 with distilled water.

Duplicate 1 ml. aliquots of the tissue homogenates and of the 1:5 diluted serums were assayed for sucrase activity according to the method previously described for intestinal sucrase (ref. Sec. II). However, for this experiment the incubation time was extended to one hour, and the cloudy solutions, which were sometimes obtained after color development, were centrifuged for ten minutes at 2,000 r.p.m. Blanks containing 1 ml. of the diluted serum or of the tissue homogenate plus 1 ml. of distilled water instead of 11% sucrose were also tested in conjunction with each sample.

3. RESULTS

The results of this survey of the sucrase activity in rat tissues other than the wall of the small intestine are presented in Table XIX. Data from Table V for the third 10 cm. section of the small intestine are also included for comparison.

Of all the tissues examined, only the sections of the small and large intestine exhibited sucrase activity. In the latter tissue the sucrase activity in the five sections examined was 0.00, 1.93, 1.91, 4.60, and 8.60 units per gram of wet tissue respectively.

Table XIX
The Distribution of Sucrase
in the Tissues of Normal Adult Rats

Tissue or Organ	No. of Animals	Sucrase Activity in Units per Gram of Wet Tissue (15 minutes incubation)*
Brain (cerebral hemispheres)	5	-
Lung (right)	3	-
Heart (whole)	4	-
Blood serum	5	-
Kidney (right)	5	-
Spleen	2	-
Thigh muscle (right leg)	3	-
Stomach: (a) Cardiac region	4	-
(b) Pyloric region	5	-
Pancreas	5	-
Caecum (4 $cm.^2$ section)	4	-
Large intestine (first 5 cm. section)	5	3.01 ± 1.50
Small intestine (third 10 cm. section)	10	43.7 ± 2.22

*The sucrase activity for the large intestine is calculated for the 15-minute incubation period from the values obtained after an actual experimental incubation period of 1 hour.

4. DISCUSSION

The results of the survey carried out indicate that the distribution of sucrase activity in the rat is similar to the distribution of the enzyme in the tissues of other vertebrates.

The finding of traces of sucrase activity in the large intestine, but not in the caecum, is somewhat disturbing. If the enzyme in the former is derived from the small intestine by seepage, or by adhesion to the feces, activity should also be present in the caecum. However, this was not the case, and, furthermore, the relatively large mount of sucrase activity associated with one of the large intestinal sections examined seems to be much greater than any activity that might be derived by seepage or by adhesion. The possibility of bacterial action must also be considered since fecal material could not be entirely removed from the cecal and colonic sections by washing. Intestinal bacteria such as Escherichia coli and streptococci may exhibit sucrase activity (19, 69). However, no bacterial growth was observed in any of the homogenates, and, in addition, bacterial action would also have been associated with the cecal sections as well as with those of the colon. The amount of fecal contamination would, of course, greatly influence the number of bacteria present.

In consideration of the experimental data and of the role of the factors mentioned above, the most probable source of the sucrase of the large intestine is the glands of the mucosa. The crypts of Lieberkühn occur both in the caecum and large intestine of mammals as well as in the small intestine (31). This distribution of the crypts is the same in man (46) as in the lower mammals.

In the rat, sucrase activity is associated largely, if not entirely, with the intestinal tract, most of the activity being concentrated in the

first half of the small intestine. Also, one may conclude that sucrase performs a solely digestive role in the animal.

5. SUMMARY

1. Various tissues of normal adult rats were examined for sucrase activity.

2. Sucrase activity was associated with only the small and large intestines. The activity was very low in the latter, but, nevertheless, it is thought to be a product of the glands of the mucosa.

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